THE ACTINOMYCETES

VOLUME 3

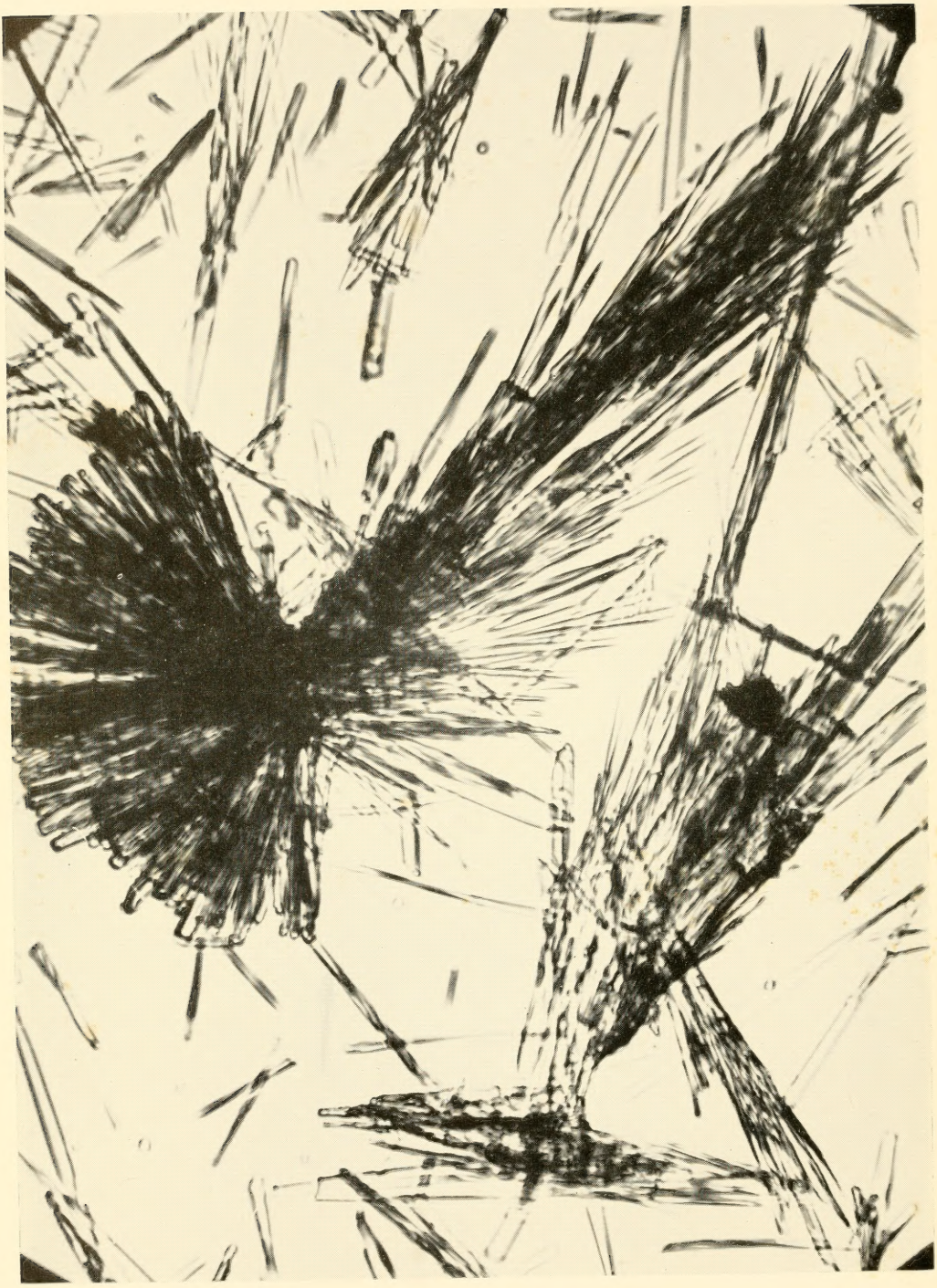
ANTIBIOTICS OF ACTINOMYCETES

Selman A. Waksman

H. Lechevalier

THE ACTINOMYCETES

VOLUME III



Crystals of Candidin

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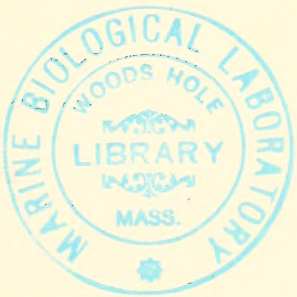
by
Selman A. Waksman
and
Hubert A. Lechevalier



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PREFACE

In 1945, the senior author published a book entitled "Microbial Antagonisms and Antibiotic Substances," which presented a comprehensive review of the knowledge of this subject at that time. Of the antibiotics listed, only six had been isolated from cultures of actinomycetes. Only one of these, namely streptomycin, had found at that time practical application in the treatment of human and animal diseases. A second edition of the book appeared in 1947. The number of antibiotic preparations obtained from cultures of actinomycetes did not increase appreciably during this brief period.

In 1953, in a volume entitled "Guide to the Identification and Classification of the Actinomycetes and their Antibiotics," we described more than a hundred chemical compounds and preparations isolated from cultures of actinomycetes. About ten of these had found practical application in chemotherapy.

The publication of the present volume, only 8 years later, emphasizes the tremendous progress made in this field. More than 400 substances and preparations have been isolated. More than 30 have found practical application in chemotherapy.

No attempt has been made in this treatise to cover the very extensive literature on the isolation, identification, and utilization of antibiotics of actinomycetes. Only the more pertinent references to the developments of the subject are presented in this volume. For a more detailed analysis of the background of our knowledge of the antagonistic relationships among microorganisms and the formation of antibiotic substances, especially by species of actinomycetes, the reader is referred to the 1945 and 1947 volumes. Detailed literature references are best found in the special treatises dealing with individual antibiotics.

The authors wish to express their sincere appreciation to Mrs. Mary P. Lechevalier for her unselfish and painstaking efforts in the assimilation of most of the literature for Part B of this volume. They are indebted to Dr. Maxwell Finland of Boston City Hospital for reviewing Chapter 8; to Dr. Vernon Bryson of the Institute of Microbiology for reviewing Chapter 10; and to Dr. O. K. Saz of the National Institutes of Health for reviewing and suggesting various corrections and changes in Chapter 9. We also wish to thank Dr. E. Borowski and Dr. N. Gerber for many suggestions helpful in preparing Chapter 6. The editorial assistance of Mr. R. A. Day and Mrs. H. B. Kitchen and the secretarial assistance of Mrs. R. Nehlig are gratefully acknowledged. Some of the necessary translations were kindly supplied by Dr. Nobuo Sakurai, Dr. Tadashi Arai, and Miss Sandra Czaplicki.

Selman A. Waksman
Hubert A. Lechevalier

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INTRODUCTORY

Detailed studies of the microbiological population of the soil have revealed not only the presence of numerous actinomycetes but also the fact that the growth of certain of these organisms exerts a depressive effect upon the growth of other microorganisms, notably bacteria and fungi. Casual observations of cultures of actinomycetes isolated by Gasperini from 1892 to 1895, Müller in 1908, Greig-Smith from 1911 to 1917, Lieske in 1921, Gratia and Dath from 1924 to 1927, and Rosenthal in 1925 further demonstrated that these organisms have the capacity to produce chemical substances, now known as antibiotics, which inhibit the growth of other organisms. That these observations were not isolated instances but were characteristic of a large number of the actinomycetes was also established in the various surveys carried out by a group of Russian investigators from 1935 to 1939.

Systematic investigations of the effect of actinomycetes upon other soil organisms, carried out in our own laboratories since 1935 (Waksman, 1937, 1941, 1947), resulted in 1940 in the isolation, in crystalline form, of a pigmented antibiotic which was named actinomycin. This was followed by the isolation in our laboratories of streptothricin in 1942, micromonosporin and streptomycin in 1943, grisein in 1946, neomycin in 1948, and later of a number of other antibiotics, notably streptocin, ehrlichin, fradicin, candicidin, and candidin. Some of these antibiotics, especially streptomycin and neomycin, have found extensive practical application in the control of numerous human, animal, and plant diseases; more recently actinomycin was shown to possess activity against certain forms of cancer; candicidin and candidin give promise as antifungal agents. Nearly all of them are of scientific interest. Numerous other antibiotics soon were isolated in various other laboratories throughout the world.

In recent years, the field of antibiotics has undergone spectacular developments. The ever growing importance of these compounds in the control of human and animal diseases as well as of certain plant diseases, in animal nutrition, in food preservation, in the preservation of biological materials, and in other fields of human endeavor has revolutionized medical practice and many of the habits of modern life. The antibiotics have added untold wealth to our economy and have resulted directly in the prolongation of millions of human lives. They have also introduced a new concept in our understanding of microbial life in natural environments and have added greatly to our understanding of certain chemical reactions in biological systems.

These developments in the field of medical science and the important practical applications in agriculture are due primarily to specific biological and chemical properties of the antibiotics, particularly their antimicrobial activities. Among these, their selective destructive action against various microbial pathogens and their relative harmlessness to the hosts attacked by the pathogens are of particular significance. Antibiotics affect various microbes at different rates. They are not generalized antiseptics and disinfectants. Each antibiotic is characterized by a selective antimicrobial spectrum, or the ability to inhibit the growth of or destroy certain microbes but not others. The various microbes differ, moreover, in the degree of their sensitivity to each antibiotic. On prolonged contact with a given

antibiotic, microbes usually become resistant to this antibiotic as a result of the selection, by the antibiotic, of resistant mutants.

The potentiality of a particular antibiotic for important therapeutic usefulness in the treatment of one or more infectious diseases depends largely upon its action on the causative agents of the disease and its lack of toxicity to the affected animals or plants. There are very few infectious diseases now known, aside from those caused by viruses and certain protozoa and some fungi that are not completely or partly controlled by the use of antibiotics. Nearly all the diseases caused by bacteria, and some of the diseases caused by fungi, rickettsiae, and the psittacosis-lymphogranuloma group of organisms, certain amoebae, and trichomonads, lend themselves readily to antibiotic therapy. Such diseases include not only those that afflict man, but also those that attack animals and plants.

Some antibiotics also possess a marked growth-promoting effect upon animals and have thus found practical application in the nutrition of these animals. This is true especially of nonruminant animals, such as swine and poultry. Ruminant animals are usually excluded, since antibiotics may affect adversely the bacterial population of the rumen which assists such animals in the digestion of cellulosic food materials; but even some of the ruminant animals may benefit at a certain stage of their development from the use of antibiotics. Antibiotics at low concentrations may exert a growth-promoting effect also upon certain microbes. This effect becomes growth-inhibiting and even destructive at higher concentrations; hence, the concentration in which antibiotics are used is of prime importance.

Because of their specific selective action upon microbial cells, antibiotics are ideal agents for the preservation of semen, virus preparations, vaccines, and similar biological materials. Recently, antibiotics have been utilized for the preservation of foods, especially poultry and certain vegetables. Since a single antibiotic will not inhibit all forms of microbial life, more than one antibiotic may be required. Before the food is eaten the antibiotics must be inactivated, as by boiling, since their constant consumption in the food might exert dangerous effects upon the human body.

The potentialities in the utilization of antibiotics in the life of modern man are still far from exhausted. Wherever man has had to combat microbes—be they injurious to his own health or to that of his herds and crops or be they destructive to his industrial products or to his foodstuffs—he has found and will continue to find in the antibiotics a source of great assistance.

However, as with every other great discovery that has revolutionized human life, new problems have arisen as a result of the usage of antibiotics. The prevalence of certain microbes resistant to specific antibiotics is now a major problem of chemotherapy. As resistant forms appear, new antibiotics, or new forms of known antibiotics, have to be found to eradicate them. This seems to be an endless process. The problem might be minimized by a rotation program in the usage of antibiotics. The disturbance in the microbiological equilibrium existing in nature by the extensive use of chemical agents that tend to eliminate certain members of the microbial population and not others may not only stimulate the development of resistant strains but may also lead to the appearance of undesirable mutants of microbes. One should consider, further, the dangerous potentialities of reduced natural resistance in the human and animal body as a result of the elimination of infectious organisms before the body has had a chance to react immunologically. And finally, we must not forget that antibiotics have contributed many problems to geriatrics by greatly increasing the average life span of man.

In tracing the history of antibiotics, which brought about a revolution in medical science and clinical practice, the future historian will no doubt designate the years 1939 to 1940 as a turning point in the history of medicine and of microbiology as well. In those years began a period which has already been designated, medically speaking, as the Age of Antibiotics. The actinomycetes have played a dominant part in this development. With the single exception of penicillin, they have yielded the most important antibiotics now used in medicine, in veterinary science, and in animal nutrition. Annual production of antibiotics, mostly produced by actinomycetes, has reached the colossal figure of 2.5 million pounds in the United States alone.

In addition to streptomycin and neomycin, the actinomycetes have contributed chloramphenicol, the tetracyclines, the erythromycins, the novobiocins, and the polyenes, to name only a few. Numerous industrial plants in this country and abroad are concerned with the manufacture of antibiotics produced by actinomycetes. Hundreds of laboratories throughout the world are engaged in the search for new antibiotics active upon diseases not subject to control at the present time; in this search, actinomycetes and their antibiotics play a dominant part.

The story is still far from complete. In presenting this summary, the authors hope to coordinate information that has been accumulating so rapidly that even the experts have had difficulty in absorbing and assimilating it.

Part A

Nature, Formation, and Activities of Antibiotics Produced by Actinomycetes

Chapter 1

Microbial Antagonisms and Production of Antibiotics

Living organisms are not found in nature in separated pigeonholes but are in constant association with one another. During these contacts various types of reactions can be noted. Even though these interactions are often difficult to classify, the following types can be recognized: (1) symbiosis, (2) competition, (3) predatoriness, (4) parasitism, and (5) antagonism. In order to simplify the following discussion, these interactions are considered as if they occurred only between two individual organisms or groups of organisms.

Symbiosis

The word *symbiosis* is used to designate the harmonious relationship between two organisms which is beneficial to both partners. In the world of microorganisms there are many examples of symbiosis.

The lichens represent a symbiotic association between an alga and a fungus so harmonious that it forms physiological and morphological types which are different from either fungi or algae. Either one or the other may be the dominant partner, that which encircles the weaker partner and is mainly responsible for the shape of the lichen. In most cases the fungus is the dominant partner. The fungal mycelium penetrates the substratum on which the lichen grows, be it rock, bark, wood, or soil, and secretes acids and enzymes that dissolve and break down the substratum, often with a resulting beneficial nutritional effect. The entrapped alga furnishes the products of its photosynthesis.

Mycorrhizas are structures formed by interaction of the mycelium of certain fungi and the roots of certain higher plants. The nature of the interrelationship between the roots and the fungi is still the subject of numerous studies, but it seems obvious that the association is beneficial to the plants. The fungus probably helps the plant by absorbing water and nutrients from the soil and benefits from the association by receiving food from the plant. In some instances the association is so successful that the plant produces no chlorophyll and depends on the fungus as a universal provider. This is true of the *Ericaceae*, *Monotropa* (Indian pipe), and the orchid *Corallorrhiza*. In nature, all orchids depend on fungi for their very life even though most of them have chlorophyll. In the laboratory, orchid seeds can be germinated free of mycorrhizal fungi if provided with sucrose which has been autoclaved at an acid pH. It is a fair assumption, then, that fungi furnish orchid seeds with a mixture of sugars and sugar degradation products.

Symbiosis between animals and microorganisms is represented by certain scale insects and a fungus belonging to the genus *Septobasidium*. The insect feeds by sucking on a plant, the fungus sending hyphae inside the insect and growing luxuriantly on

the outside, forming a protective mat. This fungal mass is inhabited both by mycelium infected scale insects, the only function of which consists in feeding the protecting fungus, and by noninfected scale insects which take care of the reproduction. In this case protection is traded for food. Further information on the phenomena of symbiosis in nature is found in the work of Vuillemin (1889), Waksman (1937), Christensen (1951), and Gaullery (1952).

Competition

Different living organisms may feed on the same substances, with a resulting conflict. Obviously, in nature, microorganisms capable of utilizing the same food will compete for whatever concentrations of that food are available. If two organisms can utilize the same nutrient with the same ease, other factors will regulate which one of the two will have the supremacy. For example, at high temperature, thermophilic organisms will be favored; in the absence of free oxygen, anaerobic organisms will be favored.

Predatoriness

Any living organism that consumes another living organism is a predator. Examples of such an association are most common among animals but are not restricted to the animal kingdom. Carnivorous plants with specialized leaves capture insects. The leaves of *Sarracenia* and *Nepenthes* are shaped like an urn and are filled with a liquid, diluted by rain water. If an insect is trapped in the urn, the motion of the insect starts the secretion of proteolytic enzymes, the pH of the liquid becoming acid. Bacterial action is not essential for the decomposition of the insect, since the sterile liquid has definite proteolytic properties. In other plants, such as *Drosera*, the insect is trapped by mucilaginous tentacles which can also produce proteolytic enzymes. In plants of the genus *Dionea*, the leaf folds along its central nervation and traps the insect in a forest of hard bristles, proteolytic enzymes being produced.

Predatoriness is common among protozoa, but even some of the fungi are predators. Some of these organisms specialize in catching nematodes; others can trap protozoa. This process has been studied extensively by Drechsler (see Duddington, 1957). Certain fungal species have developed different types of nematode traps. These may be mycelial loops strong enough to hold a nematode if the worm sticks its head through one of them. The mycelium will then invade the body of the nematode and digest it. Possibly the fungi secrete a substance which attracts nematodes and incites them to stick their necks in the loops. It is interesting to note that Pramer (1959) has shown that the nematode-trapping fungi form traps only under the stimulation of a substance found in many animal tissues including those of nematodes.

The biological significance of predatoriness is clear in the case of a fox catching a chicken but harder to explain logically in the case of higher plants and fungi. The insect-catching plants are indeed able to carry out photosynthesis and they rarely live in such poor soil that no nitrogen would be available to them. The nematode-trapping fungi are able to utilize the organic and inorganic nutrients of the soil. Still they are performing what seems to us the nonessential function of predators. It may be of interest to note here that Vuillemin first applied, in 1889, the designation "antibiosis" to phenomena of predatoriness, standing between strict saprophitism and parasitism, as a "snake devouring its prey."

Parasitism

Predatoriness differs from parasitism in that a predator destroys its prey outright, whereas a parasite usually feeds on the living host. It is common practice to differentiate between facultative and obligatory parasites. The term *obligatory parasite* may be only an

expression of our lack of knowledge of the nutrition of the organisms that we so label (Gaullery, 1952). The pathogenic actinomycetes all fall in the group of facultative parasites, as amply illustrated in Volume I, Chapters 17 and 18, and in Volume II, Chapters 2 and 3.

Antagonism

Antagonism is the phenomenon by which one living organism inhibits the growth of another one by creating an unfavorable set of conditions such as the production of toxic chemical substances. In the case of microorganisms, antimicrobial substances may be of two types: (1) chemical compounds toxic in high concentrations, such as certain acids (nitric, sulfuric, acetic, butyric, lactic, fumaric) and alcohols (ethyl, butyl); (2) chemical substances toxic in very dilute solutions. These are called antibiotics and are usually selective in their antimicrobial action, being much more active against certain microorganisms than against others.

The phenomena of antagonism among microorganisms, notably among actinomycetes and bacteria, have been examined in detail by Greig-Smith (1917), Millard and Taylor (1927), Alexopoulos and Herrick (1942), Waksman (1937, 1945, 1947), Florey, Chain *et al.* (1949, 1952), and Řeháček *et al.* (1960). The ecological aspects of microbial antagonisms have been reviewed by Brian (1957) and Waksman (1961). In discussing the phenomena of symbiosis and antagonism, one cannot overlook the phenomena of adaptation (Stanier, 1953).

Definition of an Antibiotic

The word *antibiotic* is now an integral part of the vocabulary of the layman as well as of the scientist and the medical man. Like any term that is employed widely, however, the word is often used loosely.

As pointed out previously, the word *antibiosis* was used in 1889 by Vuillemin to describe a type of association in which one living creature was destroying another in order to sustain its own life. This broad concept changed in time, and Papacostas and Gaté (1928) limited the meaning of the word in their review of the problem of bacterial associations. According to them, when one organism was exerting an injurious effect upon another *in vitro*, the type of association should be called "antibiosis"; when the same phenomenon occurred *in vivo* the association should be called "antagonism."

The noun *antibiotic* was introduced by Waksman in 1942 (1947) to designate a chemical substance of microbial origin which had the property to inhibit the growth of microorganisms. Waksman in 1947 published the following definition of the word: "An antibiotic is a chemical substance, produced by microorganisms, which has the capacity to inhibit the growth and even to destroy bacteria and other microorganisms." Benedict and Langlykke, later in 1947, modified this definition to comprise substances which act upon certain organisms at least, in very dilute solutions. This qualification avoided the inclusion among antibiotics of such products of microbial metabolism as acetic acid and ethyl alcohol. Waksman recognized the validity of this argument and corrected his original definition in 1951.

Several workers suggested that the word *antibiotic* should not be limited to substances produced by microorganisms. They felt that the use of a specific term for the product of microorganisms would seem to imply that microorganisms have a special property that other organisms do not have. Since higher plants and animals are known to produce substances similar to and in certain cases identical with antibiotics, why not apply the term to all substances of biological origin which have the aforementioned properties?

Mascherpa (1954) proposed the following definition: "Antibiotics are substances spontaneously produced by living organisms (or synthetically obtained, but with analogous structure to that of natural products) en-

dowed with selective antibacterial action through antimetabolic mechanism." Umezawa (1956) suggested the inclusion among antibiotics not only of substances of microbial origin but also those produced by higher forms of life; their action should not be limited to only microbes, but should also include tumors. The definition thus becomes: "Antibiotics are chemical substances that are produced by living organisms and that have the capacity to inhibit the growth of microorganisms or other living cells."

Words are but a means of conveying ideas from the mind of one person to another. The chief requisite is that the meaning of the word be clear to everyone.

We feel that the word *antibiotic* should be used in its original meaning. An antibiotic is not, however, a unique type of substance; it can be an antibiotic and something else at the same time. For instance, chloramphenicol is an antibiotic and also a synthetic chemotherapeutic agent, since it can be synthesized chemically. Citrinin is an antibiotic from *Penicillium citrinum* and also an antibiotic-like substance produced by a plant.

The word *antibiotic* does not imply any specific type of action of the substance so long as the effect is produced by minute concentrations. Certain antibiotics act in an indirect fashion. For instance, penatin, produced by certain penicillia, is toxic to microorganisms because of its enzymatic liberation of hydrogen peroxide.

Antibiotic Production in Soil

Although antagonism caused by the production of antibiotics is easily demonstrated under the artificial conditions of laboratory culture, it is rather difficult to demonstrate that production of antibiotics by soil microorganisms in natural soil does occur. This is complicated by the fact that most of the antibiotics are readily destroyed by some of the microorganisms present in the soil.

One method used to study this phenomenon consists in inoculating soil with known antibiotic-producers and, after a period of incubation, attempting to detect the particular antibiotic. Four different types of experiments have thus been performed. The antagonist is inoculated: (1) into sterile soil supplemented with various nutrients, such as sugars, proteins, and peptones; (2) into sterile unsupplemented soil; (3) into unsterilized soil with supplemented materials; and (4) into unsterilized unsupplemented soil.

Demonstration of the production of an antibiotic in sterile soil supplemented with organic nutrients is easy, since the environment is highly artificial and not very different from that of pure culture studies in laboratory media (Siminoff and Gottlieb, 1952). In sterile unsupplemented soils, few antibiotics have ever been produced.

In the presence of a normal soil microflora, demonstration of the production of a given antibiotic in soil becomes most difficult because members of the microflora compete for food with the artificially added antagonist and, moreover, if the antibiotic is formed, it is commonly microbiologically degraded. Investigators have surmounted these difficulties by adding massive inocula of the antagonist under study to nutritionally favored loci in the soil. The production of known antibiotics in normal soil, in loci rich in organic matter, was demonstrated by inoculating organic materials such as straw and seeds of higher plants with the known antibiotic-producers and burying them in soil. After a period of incubation, proper extraction techniques revealed in certain cases the production of the antibiotic under investigation. This method was successful mainly in the study of fungal antibiotics (Jeffreys *et al.*, 1953; Wright, 1956).

From these experiments we can conclude that the production of antibiotics, as we visualize it in the laboratory, does not occur

in nature. The useful antibiotics, which are mainly the product of the metabolism of actinomycetes, fungi, and bacteria, are not natural products in the same sense that quinine is. It is possible to collect bark from *Cinchona* trees in naturally occurring stands and to extract from this bark commercially useful quinine. It is not possible to extract soil and isolate from the extract chloramphenicol or any of the other important antibiotics. Antibiotics as we know them are laboratory products obtained by growing pure cultures of microorganisms under nutritionally rich and well aerated conditions not to be found in the soil.

The difference between the ease of extracting quinine from a tree grown under conditions unaltered by man and the impossibility of extracting chloramphenicol from *Streptomyces venezuelae* naturally occurring in a normal soil may be only a reflection of the differences in the physical size of the tree, the actinomycete, and man. The actinomycete in a natural soil sample is indeed a needle in a haystack.

The possibility then remains that small amounts of antibiotics may play a role in the microcosm that surrounds the hyphae of the antibiotic-producing organism. These antagonistic interactions would probably be more pronounced in the vicinity of accumulated organic matter. At present, as can be seen from the excellent discussion of Brian (1957), too little solidly grounded information is available to warrant much more than speculation about the ecological significance of antibiotic production.

Production of Antibiotics by Actinomycetes

Be that as it may, the actinomycetes are prolific antibiotic-producers in the laboratory and the factory. The compilation of antibiotics of actinomycetes in Part B of this book includes the listing and description of some 400 chemical substances and preparations. Most of these were found in the culture broths of various organisms and a few in the mycelium. These substances and preparations vary greatly in their physical and chemical properties, antimicrobial activities, toxicity to animals, and chemotherapeutic potentialities.

About 30 antibiotics produced by actinomycetes have already found extensive application in the treatment of various human and animal diseases. These include streptomycin and its derivative dihydrostreptomycin, chloramphenicol, the tetracyclines, viomycin, neomycin, cycloserine, erythromycin, novobiocin, oleandomycin, kanamycin, vancomycin, cycloheximide, nystatin, amphotericin B, trichomycin, and paromomycin. They are used in the treatment of a great variety of infections caused by gram-positive and gram-negative bacteria, mycobacteria, rickettsiae, the members of the psittacosis-lymphogranuloma group of intracellular parasites, trichomonads, amoebae and other protozoa, monilia and other fungi. Some antibiotics, such as streptomycin and cycloheximide, have found application in the treatment of plant diseases. Some are used in animal feeding. Some, like the tetracylcines, have found application in the preservation of poultry and certain other foodstuffs. This wide range of usefulness makes the actinomycetes the most important of all the antibiotic-producing microorganisms. Only four fungal products (penicillin, fumagillin, variotin, and griseofulvin) and three bacterial products (bacitracin, polymyxin, and tyrothricin) are used in medicine.

Chapter 2

How Antibiotics Came to be Recognized

Anyone attempting to analyze the historical background of our present day knowledge of antibiotics must take into consideration not only scientific concepts, but also popular observations and beliefs. Moreover, there has always been the danger of reading into observations the results of subsequent experiments. With our present knowledge, we are now able to analyze complicated observations of the past in simple terms, but we may forget that the pioneer observer was not thinking along the same lines that we now are. It is a further problem to decide whether one observation truly exerted an influence upon a subsequent line of scientific development, since this is largely a matter of interpretation. Was it merely a name proposed at the right time, or was it the persistence of one or another investigator that turned a particular observation into an important scientific or practical contribution?

Even though we have hindsight at our disposal, we cannot always analyze faithfully the events of the past, but we can try.

1. One may first consider the origin of our knowledge of penicillin. The French bacteriologist Duchesne wrote a thesis, published in 1897, on the antagonisms between fungi and bacteria. He described experiments in which injection of large amounts of a culture of *Penicillium glaucum* permitted the survival of guinea pigs that had received lethal doses of gram-negative bacteria. He concluded: "One might thus hope that by pursuing the study of biological competition between molds and microbes, one might be led to the discovery of other facts which would be directly useful and applicable to prophylactic hygiene and to therapy." Had he lived long enough or had others appreciated the significance of his work, the discovery of penicillin might have come at a much earlier date. Accurate observations on the production of bacteriolytic agents by "a strain of *Penicillium glaucum*" were made in 1925 by the Belgian bacteriologist Gratia (1930). Had he used a specific name ("The trouble with you, my boy," said the famous immunologist Bordet of Brussels to his former student Gratia of Liege, "is that you do not christen your babies.") for the metabolic product of his *Penicillium* culture that exerted an inhibiting effect upon bacteria, the now famous antibiotic might have been known under a totally different label. Finally, Chain and Florey (1940) of Oxford University, England, after having studied various natural products, including the lysozyme of Fleming (1922), turned their attention in 1938–1939 to the antimicrobial substances produced by microorganisms. They isolated from a culture of *Penicillium notatum* the active antibacterial substance observed by Fleming in 1928 (1929) and

designated later by him as "penicillin," and demonstrated its effectiveness in the treatment of human diseases. Thus was culminated the historical background of the most useful of the antibiotics. With hindsight we can say that, as was true of the sulfa drugs, penicillin could have been discovered sooner.

2. An even older antibiotic, pyocyanase, offers another illustration. This antibiotic was extensively studied for nearly half a century. It failed, however, to achieve practical use because it was a highly complex and somewhat toxic compound. Had another organism producing a more desirable substance been used, the field of antibiotics might have been opened much earlier.

3. The production of antibacterial substances by aerobic spore-forming bacteria has been known for many years. It was investigated in detail by Nicolle in 1907, by Pringsheim in 1920, and by Much and Sartorius in 1924–1925, to name only a few. All this work failed to receive the attention it deserved. It took some years before this problem was taken up again in a more systematic manner by Dubos (1939), who successfully isolated in 1938 a group of active substances known as tyrothricin, thus opening the field of the antibacterial antibiotics.

4. The French clinician Vaudremer reported in 1913 that the mold *Aspergillus fumigatus* exerts a marked antitubercular effect. The extracts of this mold were used in the treatment of 200 tubercular patients with varying degrees of success. Had the potentialities of such a method of treatment been more clearly visualized, screening methods might have been established, the study of chemotherapy of tuberculosis might have been initiated, and perhaps the problem might have been solved much earlier; the world would not have had to wait nearly three decades longer before streptomycin was isolated and its antitubercular properties were established.

5. Lieske (1921) in Germany, Krassilnikov and Koreniako (1939) and Kriss (1940) in Russia, Gratia and Dath (1924) in Belgium, and Rosenthal (1925) in France, among others, recognized that cultures of the various actinomycetes possess marked antibacterial properties. The agents studied by these investigators were often bacteriolytic. The concept of a nonlytic antibiotic was not very clear. Gratia and his collaborators were able not only to experiment on animals with the therapeutic powers of preparations obtained from an actinomycete (*Streptothrix albus*), but even to treat with success human patients suffering from staphylococcal infections. The methods were quasi-immunological. The lytic properties of the actinomycetes have been associated with the phages of d'Herelle and with the lysozyme of Fleming, and to this day the literature on the actinomycetic agent of Gratia, later named *actinomycetin* by Welsch (1937, 1942), tends to be rather confusing.

6. Whereas most of the above observations were largely concerned with the antibacterial properties of actinomycetes, Müller (1908) and, more recently, Alexopoulos and Herrick (1942) demonstrated that as many as 38.8 per cent of such cultures were also effective against fungi.

The opening of the field of the antibiotics of actinomycetes was delayed until 1940, when actinomycin, a substance simpler than actinomycetin, was isolated in our laboratories. Here again, a lack of foresight on our part should be noted. We understood the concept of antibiosis, but we did not foresee the potentialities of actinomycin as an antitumor agent. This discovery had to wait for the investigations of Stock (1950), Hackmann (1952), and numerous others.

Despite this fumbling, antibiotics became a part of our lives, following the route that we now will trace.

Early Observations

The earliest observations on the effect of microbial products upon disease came before microbes were recognized and their metabolic processes known. They were made long before the etiology of human and animal diseases was established.

The Bible and postbiblical writings, such as the Apocrypha, contain numerous references to the effect of the soil upon the destruction of disease-producing organisms:

> And when thou goest out of thy camp
> Take a spade with you
> And cover up what comes out of you.

There is actual reference to the presence of medicines in products of the soil:

> The Lord created medicines out of the earth,
> And he that is wise shall not abhor them.

Those who gave such advice may have had an inkling of many modern ideas which were the result of subsequent observations. Since a great many antibiotic-producing organisms inhabit the soil under our feet, the above statement may have some justification. The introduction of pathogenic microbes into the soil is known to result in their destruction and for many years was even thought to induce the development of organisms that are able to produce antibiotics. This theory has not been supported by more critical experimental evidence.

Folklore abounds in prescriptions consisting of the application of moldy cheese, rotting meat, and other moldy products to wounds to treat infections or prevent their development. Jules Brunel, in an article published in 1944, quoted the following statement from a Canadian biochemist: "It was during a visit through central Europe in 1908 that I came across the fact that amost every farm house followed the practice of keeping a moldy rye loaf on one of the beams in the kitchen. When I asked the reason for this I was told that this was an old custom and that when any member of the family received an injury, such as a cut or a bruise, a thin slice from the outside of the loaf was cut off, mixed into a paste with water and applied to the wound with a bandage. I was assured that no infection would then result from such a cut."

The North American Indians were also users of moldy products. In the eighteenth century, they were reported to have applied rotten wood to wounds to prevent suppuration.

In Europe, bakers' yeast was applied to abscesses, probably with the intention of making the abscesses come to a head more rapidly. This practice was followed by most of the reputable medical authorities, including Lieutaud, physician to Louis XVI. The yeast was also taken orally. Perhaps this was early vitamin therapy rather than early antibiotic therapy. We might even consider this as a precursor of the combined antibiotic-vitamin therapy which is now so popular!

First Experimental Observations

With the growth of scientific medicine during the nineteenth century, certain events took place that were to be the basis of modern antibiotic research. These can be briefly summarized as follows:

1. There was a growing knowledge of mixed infections and the replacement of parasites by saprophytes when the latter were introduced into infected animals.

2. Soil microbiology was born, bringing into focus the complex microbial population of the soil and the interrelations among different microorganisms in natural substrates.

3. The investigation of the effect of green manures upon the control of the potato scab organism and the effect of organic manures upon certain root rots of plants led to developments in the field of plant pathology which contributed to our understanding of

the interrelations between disease-producing and saprophytic microbes in plant diseases.

4. Direct observations were made on the effect of metabolic products of saprophytic organisms upon disease-producing organisms and upon infections.

One of the early observations, made by Roberts in 1874, is of particular interest in this connection. Certain liquid extracts in which a green mold had been growing luxuriantly became infected with bacteria only with great difficulty. The possibility was suggested that the mold held in check the growth of bacteria. On the other hand, liquids full of bacteria did not favor good growth of the mold (*Penicillium glaucum*). An antagonism was also observed between the growth of different races of bacteria. Roberts concluded, "There is probably in such a case a struggle for existence and a survival of the fittest."

In 1876, the British physicist Tyndall also reported on the growth of wild cultures of bacteria and fungi in organic infusions; he spoke of "the struggle for existence between the *bacteria* and the *penicillium*. In some tubes the former were triumphant; in other tubes of the same infusion the latter was triumphant." He concluded that "the *bacteria* which manufacture a green pigment appear to be uniformly victorious in their fight with the *penicillium*."

These early reports heralded the development of penicillin and pyocyanase.

Soon afterward, in 1877, Pasteur and Joubert noted that "... one can infect abundantly an animal with anthrax without the animal becoming diseased; it is sufficient that the fluid contain in suspension simultaneously the anthrax organism and a common or harmless bacterium." They then added prophetically, "These facts perhaps justify the highest hope for therapeutics."

Soon numerous other observations were recorded concerning the effect of saprophytic microbes upon disease-producing organisms. Cantani, for example, wrote in 1885: "The known fact that certain bacteria can destroy the cultures of other microbes, even those that are pathogenic, if they come into contact with them in any way, gave me the idea of exploring this procedure for the treatment of various infectious diseases." He treated pulmonary tuberculosis by introducing by nebulization the saprophyte *Bacterium termo* into the lungs of a patient. Even though he reported clinical improvement, the method was never used on a large scale.

The very same year, Cornil and Babès wrote: "The study of the reciprocal action that bacteria have one upon another, persisted in, and enlarged in scope, might lead to therapeutical results." These authors are also credited with introducing the agar cross-streak test. This test was further developed by Garré, who showed in 1887 that strains of *Pseudomonas* were producing a specific diffusible substance which was able to inhibit the growth of staphylococci and other pathogens.

Two to three years later a number of papers were published which focused attention on the great potentialities of *Pseudomonas aeruginosa*. Bouchard, Charrin and Guignard, Kitasato, Woodhead and Wood, and Blagovestchensky all demonstrated the antibiotic powers of this bacterium against the anthrax bacillus and other pathogens. Honl and Bukovsky, in 1899, treated with an ill-defined extract of *Pseudomonas aeruginosa* more than 100 patients who had infected wounds. The results were good. In the same year, Emmerich and Löw reported their work on pyocyanase, also an ill-defined extract of the same bacterium. Pyocyanase was bactericidal and bacteriolytic (references to above papers are found in a paper by Lagodsky, 1951).

Before the turn of the century, attention

was attracted to the antibiotic potentialities of actinomycetes. Gasperini, in 1890, reported that the organism *Streptothrix Foersteri*, isolated as a contaminant, was able to lyse bacteria and fungi. It was also before the turn of the century that the first antibiotic was isolated in a crystalline form. From a strain of *Penicillium*, Gosio, in 1896, isolated and crystallized the antibiotic which was later called mycophenolic acid.

Laying the Foundation for Antibiotic Research

The first four decades of the present century were characterized by marked advances in chemotherapy. First came the arsenicals that were shown to have activity against trypanosomes. Ehrlich and his collaborators made a great number of chemical derivatives of the first compound, atoxyl, in the hope of finding a substance that would retain the antimicrobial activity of atoxyl but be less toxic to the animal body. In 1909, the 606th compound was found to possess the desired properties and was named salvarsan.

Starting from the fact that certain azo dyes were known to have bactericidal effects *in vitro*, a group of chemists from the Bayer laboratories in Germany synthesized a large number of these compounds. These were tested by Gerhard Domagk in mice infected with hemolytic streptococci. In 1932, one of the dyes, prontosil, was found to be effective *in vivo*. Strangely enough, it was active only *in vivo*. This was explained in 1935 by Tréfouël and his collaborators at the Pasteur Institute. They showed that the dye was split in the body, that sulfanilamide, one of the products formed, was the active portion of the molecule and that it was active both *in vitro* and *in vivo*. Hundreds of derivatives of sulfanilamide were subsequently synthesized and tested in animals (references to the above papers are given by Albert, 1951).

The extensive testing of arsenicals and sulfa drugs led to the development of microbiological, pharmacological, and pharmaceutical methods necessary for the progress of antibiotic research.

During the first four decades of the twentieth century, while spectacular work was being done in the field of chemotherapy with arsenicals and sulfa drugs, numerous observations, similar to those made during the nineteenth century, were published in the field of microbial antagonism. Only the most important developments will be discussed here.

The pyocyanase of Emmerich and Löw (1899) was studied in experimental animals and in human patients. For a short time commercial preparations of pyocyanase were available in Germany and were used mainly topically. The theory that pyocyanase was an enzyme did not last long, since lipoidal, heat-stable fractions of the crude extract were found to have antibacterial action.

The fungal antibiotic isolated in pure form by Gosio in 1896 was studied again in 1913 by Alsberg and Black, and later by Clutterbuck *et al.* (1932, 1933). This antibiotic, the first to be isolated in a pure form, is now virtually unknown.

In any scientific climate, the minds of men are molded by the prevailing ideas of the time. During the first four decades of the twentieth century, emphasis on enzymes and lytic phenomena was to hinder the development of antibiotics. This is true of the discovery of the Twort-d'Herelle phenomenon in 1915–1916. As mentioned previously, Gratia and his collaborators in Belgium had studied the bacteriolytic properties of actinomycetes. It should be stressed that these authors were probably the first to use the product of an actinomycete in human therapy. The treatment seems to have been used mainly for staphylococcal infections and consisted of injecting

bacteriophages in combination with what was then called "mycolysates of staphylococci." These mycolysates were the products of the lytic action of an actinomycete on the pathogen. This actinomycete was identified as *Streptothrix albus* and the antibacterial-bacteriolytic complex formed by this organism was named actinomycetin by Welsch in 1937. In a paper published in 1930, Gratia said: "We have used this association of the bacteriophage and of mycolysates of staphylococci in a very great number of cases and we believe that we may say that it is today the most effective treatment of staphylococcal infections. . . ."

Fleming, working at St. Mary's Hospital of London, had been interested first in the cellular aspect and then in the humoral aspect of immunity. He studied the bacteriolytic properties of nasal and ocular secretions. In 1922, he gave the name lysozyme to a bacteriolytic enzyme abundantly distributed in nature. Lysozyme was later found to be a mucopolysaccharidase which now plays an important role in studies of bacterial biochemistry. In 1929, Fleming published a paper on the antibacterial action of a strain of *Penicillium*. Since the culture filtrate of the *Penicillium* was mainly active against gram-positive bacteria, Fleming considered that it could be used for the making of selective media for the isolation of gram-negative bacteria. He named this active filtrate penicillin. A few more papers were published on this crude substance, but they attracted little attention. In 1932, Clutterbuck, Lovell, and Raistrick published a paper on the metabolic products of the *Penicillium* of Fleming, which was properly identified by Charles Thom as *P. notatum*. They found a chemically defined medium on which it grew and produced the antibacterial substance, which proved to be markedly unstable. Their concluding sentence, "The investigation of the isolation and chemical nature of penicillin is being continued," was almost the epitaph of Fleming's penicillin!

The Discovery of Antibiotics

The year 1939 was a milestone in the history of the world. The Second World War had started, and as the guns and the bombs began to settle human differences, scientists and physicians were faced with the problem of salvaging what they could of the mangled flesh.

An impetus was thus given to the search for antimicrobial agents, but this effect was not to be felt immediately in 1939. This date is nevertheless a turning point in the history of chemotherapy. It was about then that the work in Dubos' laboratory at the Rockefeller Institute yielded tyrothricin, and research in the New Jersey Agricultural Experiment Station yielded actinomycin. It was also at that time that Chain and Florey at Oxford University carried out their now famous evaluation of penicillin.

The new study of penicillin showed the great potentialities of this antibiotic as a chemotherapeutic drug. With the war in process, the British investigators needed help to carry out the titanic problem of producing penicillin and elucidating its chemical structure. In 1941, Florey and Heatley came to the United States. A vast research program was set up which involved the cooperation of government laboratories, such as the Northern Regional Research Laboratory in Peoria, of universities, especially the University of Wisconsin, and of many industries. Better media were developed; more active strains of penicillia were isolated; better methods (deep-tank) of culture were introduced on a large scale. Penicillin was found to be therapeutically effective and could now be produced cheaply. It opened the eyes of the world to a new type of chemotherapeutic agent, of low toxicity and of high activity, against a variety of infectious diseases caused by gram-positive

bacteria, cocci, spirochetes, and other organisms.

In 1931, Dubos and Avery incorporated polysaccharides from the capsule of type III pneumococci in soil. They isolated from the soil a bacillus which, when grown in a synthetic medium containing the polysaccharide as the source of carbon, elaborated an enzyme that specifically attacked the organism. This enzyme could protect mice from a fatal dose of type III pneumococcus. Dubos continued the soil enrichment study by introducing living bacteria into the soil, rather than the capsular material alone. From these enriched soils Dubos in 1938 isolated a strain of *Bacillus brevis* which produced an antibiotic that he called tyrothricin. In 1940, Hotchkiss and Dubos showed that tyrothricin was composed of two active antibiotics, tyrocidine and gramicidin. Tyrothricin is active chiefly against gram-positive bacteria and is used topically in medicine. The tyrothricin complex opened the field for bacterial polypeptides, to be followed soon by bacitracin, polymyxin, subtilin, and a variety of others. The practical importance of some of these is still not fully recognized. Even though it is doubtful that the soil enrichment method was a necessary step in the isolation of the active strain of *B. brevis*, the work of Dubos showed the value of systematic screening programs in the search for antibiotics.

Another blow to the chance isolation procedure of obtaining antibiotic-producing strains was dealt at about the same time by what could be called the Rutgers group of investigators. Ever since 1936, the senior author of this treatise had been interested in antagonistic relationships among soil microorganisms. Actinomycetes often showed themselves to have outstanding antimicrobial activity. In 1940, Waksman and Woodruff reported the isolation of actinomycin, the first actinomycete-produced antibiotic to be obtained in a crystalline form. After this discovery, the attention of the Rutgers group turned to products of fungi, and clavacin, fumigacin, and chaetomin were isolated. Actinomycetes were not forgotten, however, and systematic screening programs yielded, among others, streptothricin (1942), and most important of all, streptomycin (1944). Streptothricin was an interesting substance. It was basic, stable, and water-soluble; it was active against gram-negative and gram-positive bacteria, mycobacteria, and fungi *in vitro* and *in vivo*; however, it had a delayed toxicity that limited its usefulness. Streptomycin had the same general chemical properties as streptothricin but was less toxic; infections caused by gram-negative and gram-positive bacteria and mycobacteria responded to treatment with this new drug. The chemotherapy of tuberculosis was finally made possible.

The Modern Period

The success of the screening methods of the Rutgers group was partly responsible for the scrutiny of the actinomycetes in screening programs throughout the world. The results were rewarding. Antibiotics were isolated which are active not only against gram-positive and gram-negative bacteria, but also against rickettsiae and the psittacosis-lymphogranuloma group of organisms. These antibiotics include chloramphenicol (1947), chlortetracycline (1948), oxytetracycline (1950), and tetracycline (1953). Others were to come later, namely, the macrolides erythromycin (1952), carbomycin (1952), spiramycin (1954), and oleandomycin (1954). Still others, similar to streptomycin in certain respects, have found a place in chemotherapy; these included neomycin (1949), viomycin (1951), and kanamycin (1957).

Antibiotics were also found which are primarily antifungal, such as cycloheximide (1946), nystatin (1951), candicidin (1952),

trichomycin (1952), candidin (1954), and amphotericin B (1955). Others have been found which have pronounced antitumor action; these include, apart from the actinomycins, azaserine (1954), sarkomycin (1953), carzinophilin (1954), 6-diazo-5-oxo-L-norleucine (DON) (1956), and mitomycin (1956).

New antibiotics are still being discovered; most of them are being isolated from cultures of actinomycetes. Some are still being introduced into medical practice. As yet, however, no useful antiviral antibiotics have been isolated.

The uses of antibiotics have been extended far beyond their original chemotherapeutic provinces, and even into the fields of animal feeding and food preservation. Some antibiotics are used by the geneticist to select mutants of bacteria; here streptomycin occupies a place of choice. Others are used by the biochemist as specific inhibitors of metabolic reactions, such as chloramphenicol (inhibition of protein synthesis) and antimycin A (inhibition of cytochrome oxidase). The marvelous potentialities of the antibiotics have not yet been exhausted.

Chapter 3

The Search for Antibiotics: Screening Programs

The detection of antagonists capable of producing antibiotics was at first a matter of chance. It was by chance that one of the bacterial culture plates of Fleming was contaminated by the now famous *Penicillium*. Since then, investigators have tried in many ways to be more systematic in their methods of detecting antibiotic-producers.

Basic Screening Procedures

We have already discussed Dubos' enrichment of soil samples with pathogens in an attempt to increase the chances of detecting antibiotic-producers. This method would be logical if antibiotics were lytic enzymes which would have the property of making the pathogen available as food for the antagonist. Since this is not the case, the *soil enrichment method* has remained one of historical interest with no practical importance, as demonstrated in 1946 by Waksman and Schatz.

A second approach comprised efforts to *induce* antagonistic properties in a nonantagonist. Basically the method consisted in confronting a pathogen with another microorganism in a medium poor in nutrients, in the hope that the microorganism would become antagonistic toward the pathogen. Successful results have been reported by Schiller (1952) in Russia and by Davide (1949) in Sweden. Many other investigators, including us, have tried this method without success.

By far the most successful method in the search for antibiotics has consisted in testing the antagonistic properties of large numbers of microorganisms *in vitro*. The general procedure can be modified in a number of ways. Briefly, the method comprises the following steps: (1) The substrate to be studied is plated out on media which permit the growth of actinomycetes. (2) The various actinomycetes are isolated in pure cultures, usually on slants of media favorable for abundant growth of these organisms. (3) Each actinomycete culture is inoculated in Petri dishes containing agar media considered favorable for the production of antibiotics; the inoculation is usually made as a broad streak so that incubation yields a ribbon of growth of even width. (4) After growth of the actinomycete, at what is considered a favorable temperature (25–30°C) for a favorable length of time (3 to 7 days), test organisms, against which antagonists are sought, are streaked at a right angle to the actinomycetic ribbon. (5) After incubation of the test organisms under optimal conditions for their growth, the antagonistic potentialities of the actinomycetes are estimated by the width of the inhibition zone. Such cross-streak tests are illustrated in Chapter 15 of Volume I.

There is, of course, no ideal medium which permits the plating out of a natural substrate with the resulting growth of all the actinomycetes present in the substrate and

which inhibits the growth of all other microorganisms. Porter *et al.* (1960) advocate the use of an arginine-glycerol agar.

The addition of selective inhibitors permits reduction of the number of fungi or true bacteria and helps in the isolation of actinomycetes in pure cultures. Corke and Chase (1956) have used with success the antifungal antibiotic cycloheximide to eliminate fungal growth. Lawrence (1956) reduced the number of contaminating bacteria and fungi by pretreating the samples to be plated out for 10 minutes with a 1:140 dilution of phenol. The successful use of centrifugation of suspensions of substrates to be plated out, as a means of separating actinomycetes from other microorganisms, was reported by Řeháček in 1956. The suspensions were centrifuged for 20 minutes in tubes which were subjected to $904 \times g$ on the surface and $1609 \times g$ on the bottom. Under such conditions most actinomycetes were not sedimented, whereas most other microorganisms were.

Another method consists in increasing the number of actinomycetes present in a soil sample before plating out. Tsao *et al.* (1960) dried soil samples and then incubated them, buffered with calcium carbonate, in a moist atmosphere. This resulted in an increase in the percentage of viable actinomycetes in these soil samples.

For the isolation of actinomycetes in pure culture, there is, of course, no universal medium. The authors consider yeast extract-glucose agar the best general medium for this purpose. In certain countries where standard preparations of yeast extract are not available, a bakers' yeast-salt medium is a good substitute (Lechevalier and Tikhonienko, 1960).

It is always desirable to remember that the use of various other media may permit the growth of organisms which might not grow, or which might grow poorly, on yeast-glucose agar.

The media used for the actual cross-streak test must be favorable for growth of both the actinomycete and the test organisms. The ideal medium for such tests should also be free from chemicals that might inhibit the action of the antibiotics produced by the actinomycete. The selection of such a medium must, of course, be highly empirical.

TABLE 1

Cross-streak test of 269 actinomycetes freshly isolated from soil and from manure

Comparison between results obtained on nutrient agar and on yeast-glucose agar.

	Percentage of total number tested					
	On nutrient agar, width of inhibition zone			On yeast-glucose agar, width of inhibition zone		
	1–10 mm	11–20 mm	>20 mm	1–10 mm	11–20 mm	>20 mm
Mycobacterium smegmatis 607	8	10	3	7	3	2
M. smegmatis 105	8	11	3	8	7	3
M. smegmatis 7992	10	10	3	8	6	2
M. phlei 23	10	27	16	12	17	19

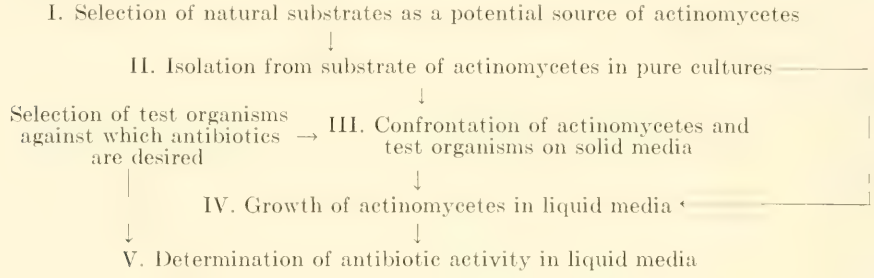
The influence of the medium used in the cross-streak test and the results obtained are illustrated in Table 1. A group of 269 freshly isolated strains of actinomycetes were tested for activity against four strains of fast growing, nonpathogenic mycobacteria on two different media, nutrient agar and yeast-glucose agar. The same amount of agar (15 ml) was used in all plates to permit comparison of the width of inhibition zones. One will note that approximately the same percentage of strains formed narrow and broad zones of inhibition on both media, but that the percentages of medium width zones were higher on nutrient agar than on yeast-glucose agar. This suggests that certain types of antibiotics responsible for the medium width zones were not formed or were inactivated by yeast-glucose agar.

Demonstration that an antagonist can produce a diffusible substance effective upon the test organisms chosen in a given screening program must be followed by demon-

stration that this substance can also be produced in liquid media. This is of prime importance, since antibiotics must be obtained in liquid media for large-scale production.

Since the production in liquid media is the chief concern of an industrial microbiologist, many large-scale screening programs bypass completely the cross-streak test and inoculate their isolates directly into liquid media, which are usually incubated on shaking machines to furnish the aeration necessary for the growth of most actinomycetes.

The following scheme outlines the basic screening procedures discussed above:



Various modifications of this general scheme have been made, and a number of special methods have been devised.

Special Screening Techniques

The crowded-plate technique, used in the early studies on the isolation of antibiotic-producing organisms, consisted in plating out heavy suspensions of natural substrates. Organisms growing on such plates, surrounded by a zone of inhibition, were selected for further work. This method did not permit the development of slow-growing actinomycetes which were submerged by bacterial growth before they had a chance to elaborate any antibiotic.

Attempts have been made to differentiate at an early stage between antibiotic-producing organisms and nonproducers by inoculating a test organism directly on plates in which the substrate had been plated out. This inoculation was accomplished either by spraying the test organism (Stansly, 1947) on the surface of the plate or by flooding this surface with a water-agar suspension of the test organism.

One of the disadvantages of these methods was that they did not permit the testing of activity against more than a single test organism. Another difficulty encountered in the screening of actinomycetes is that media selective for the growth of these organisms are often not good for antibiotic production.

An adaptation of Lederberg and Lederberg's replica plating method was described in 1953 by Lechevalier and Corke. The substrate was plated out with a dilute suspension of the natural substrate to obtain well-isolated colonies. These were transferred with a sterile velveteen stamp to a series of plates containing media thought to be favorable for the production of antibiotics. After incubation, the plates were unmolded and turned upside down. The back of each plate was inoculated with a test organism. Colonies having the desired spectrum of activity could then be isolated from the original plate from which all transfers had been made.

New techniques have been used to detect types of biological activities undetectable by previous procedures (Gause, 1958). Such was the method of Murat *et al.* (1959) which was designed to detect antibiotics active against intracellular bacteria. The method consists in allowing brucellae to be phagocytized by guinea pig monocytes. The remaining extracellular brucellae cells are killed with streptomycin. The living brucella-

containing monocytes are confronted with the antibiotic or the culture filtrate to be studied. If the antibiotic is active intracellularly, the brucellae are killed; if not, their life is detected by the formation of bacterial colonies when the monocytes are flooded with agar.

Among the special techniques, it is sufficient to mention those devised for the study of antagonists active against plant pathogens (Cooper and Chilton, 1949; Stessel *et al.*, 1953). The actinomycetes attracted particular attention because of the early observations by KenKnight (1941) and Alexopoulos (1941), among others, that they were active upon the potato scab organism and other plant pathogens.

Screening Surveys

Numerous surveys have shown that actinomycetes capable of producing antibiotics *in vitro* are widely distributed in nature. They are the most common antibiotic-producers that can be isolated from soil (Waksman and Lechevalier, 1951).

Antimicrobial Surveys

Among the previous surveys, in addition to those already listed, it is sufficient to mention those of Borodulina (1935), Burkholder (1946), Waksman *et al.* (1946), Landerkin and Lochhead (1950), Harris and Ruger (1953), and Craveri *et al.* (1960). A summary of some of both older and more recent surveys is shown in Table 2. As can be seen,

TABLE 2

Surveys of antagonistic activity of actinomycetes

Investigator	Date of survey	Total cultures tested	Percentages of cultures active against													
			Bacteria						Mycobacteria		Fungi				Viruses	
			Gram +			Gram −										
			B. subtilis	*Staph. aureus*	*Sarcina*	*E. coli*	*Ps. aeruginosa*	*P. penis*	*M. smegmatis*	*M. phlei*	*C. albicans*	Various fungi	*C. albi*	*Sacch. cerevisiae*	Bacteriophages	Newcastle disease virus
Nakhimovskaia	1937	80	59													
Alexopoulos	1941	80										56*				
Waksman *et al.*	1942	244	43													
Welsch	1942	164	46	46		0.6										
Waksman *et al.*	1946	187	36			7				65						
Burkholder	1946	7369		25		4					7					
Jones and Schatz	1947	176													28	
Cooper *et al.*	1949	2452										24†				
Landerkin *et al.*	1950	660	49	47		8	0.5									
Groupé *et al.*	1951	113														10
Rouatt *et al.*	1951	544	39	39		10	9									
Lechevalier	1951	302							21	51						
Waksman *et al.*	1952	197											48			
Asheshov *et al.*	1954	1000													20	
Aleshina and Makarovskaia	1955	1117	44					19								
Vaněk *et al.*	1958	739	52	33	63	11	8				30			28		

* Antagonistic to *Colletotrichum gloeosporioides*.

† Antagonistic to *Pythium arrhenomanes*.

gram-positive bacteria and mycobacteria are inhibited by a larger percentage of actinomycetes than are gram-negative bacteria. Fungi are also inhibited by a large number of actinomycetes. Many inhibitors of phages can be detected in the culture filtrates of actinomycetes, but their antiphage action does not show correlation with true antiviral action. As has already been mentioned, no useful antiviral antibiotic has yet been discovered.

Routien and Finlay (1952) screened thousands of soil samples obtained from widely scattered geographical areas. Certain organisms producing streptomycin, streptothricin, chloramphenicol, actinomycin, and xanthomycin-like antibiotics were extremely common; in fact, they were of worldwide distribution. Tetracycline-producing cultures were isolated only a few times. One antibiotic was obtained from only one culture isolated from one particular soil. Certain antibiotics were produced by organisms common in soils in certain localized areas. Other antibiotics came from soils collected within a restricted area. Umezawa *et al.* (1949), using the technique of Waksman and Schatz (1946) of adding a particular antibiotic (chloramphenicol) to the medium before plating out soil samples, were able to demonstrate the presence of a rather large number of chloramphenicol-producing strains, thus suggesting a wide distribution of these organisms.

Janot *et al.* (1954) isolated 7941 cultures of *Streptomyces*; 351 of these produced antibiotics. Streptothricin-producers were most common, followed by streptomycin-, then tetracycline-producers. Various cultures capable of forming framycetin (neomycin) were also found, followed by a group of nonidentified antibiotics.

Hirabayashi (1959) examined 316 cultures of streptomycetes for antiamebic activities. Nine of the cultures were found to have direct antiamebic effects. An active substance was isolated from one culture of *Streptomyces* and was named protomycin. It had no antibacterial activities. Protomycin showed inhibitory activity upon the growth of *Endamoeba histolytica* at a concentration of 25 μg per ml after 24 hours of incubation and at a concentration of 1.6 μg per ml after 48 hours of incubation. Woodruff and McDaniel (1958) emphasized that, on the average, 25 per cent of cultures of streptomycetes isolated were able to produce some kind of an antibiotic. About 90 per cent of all the antibiotic-producing cultures were found to form streptothricin or closely related compounds. At least half of the remainder produced streptomycin. Of the others, one third formed tetracyclines, and most of the rest could be identified with one of the hundreds of antibiotics which have been described from species of *Streptomyces*. In a study of 10,000 cultures isolated, about 2,500 showed antibiotic activity. All but 250 gave streptothricin-like antibiotics; about 125 formed streptomycin; 40 produced tetracyclines; and 55 yielded other previously described antibiotics. Only 30 cultures seemed to form new antibiotics.

The above calculations are based upon the examination of antibacterial antibiotics. Had a similar study included antifungal agents as well, especially the polyene type of compounds, the latter would have been found to play an important part in the distribution of antibiotic-producing properties among actinomycetes. Vaněk and coworkers (1958) found that 16.3 per cent of 739 freshly isolated strains of actinomycetes produced polyenes. Heptaenes were the most commonly produced polyenes, as reported in 1956 by Pledger and Lechevalier.

Plotho (1947) tried to correlate pigment production with antibiotic activity. A collection of 291 cultures were classified into four groups on the basis of pigments produced either in the mycelium or in the medium. Of 61 cultures (21 per cent) show-

ing activity against *Mycobacterium eos*, 21 were classified as a colorless group, 20 red-yellow, 12 red-blue, and 8 red-brown. It was suggested that use of media in which pigments could be detected readily might reveal a correlation between antibiotic activity and some other property.

Antitumor Surveys

Considerable attention has recently been focused upon the products of actinomycetes that possess antitumor activities. A number of different substances active against neoplastic cells have been isolated and described (Table 3). None of them can be said to have become cures for cancers, although some appear to offer definite promise. These substances are not comparable to antibiotics in their activities, although many appear to be definitely antibiotics, since they are also active upon bacteria or fungi. They are included in this treatise because the methods of approach to their isolation are similar to those concerned with antibiotics and also because of their selective action against different cells. These substances include actinomycin (Waksman and Woodruff, 1940), azaserine (Bartz *et al.*, 1953; Ehrlich *et al.*, 1954), 6-diazo-5-oxo-L-norleucine (DON) (Clarke *et al.*, 1956), carcinomycin (Hasoya, 1955), carzinocidin (Harada *et al.*, 1956), carzinophilin (Hata *et al.*, 1954), gancidin (Aiso *et al.*, 1954), melanomycin (Sugawara *et al.*, 1957), mitomycin (Sugawara and Hata, 1956), pluramycin (Maeda *et al.*, 1956), puromycin (Troy *et al.*, 1953), sarkomycin (Umezawa *et al.*, 1954; Hooper *et al.*, 1955), teomycic acid (Oda, 1960), and many others.

TABLE 3

Grouping of actinomycete antitumor products (Umezawa, 1956)

Pigmented substances	
Actinomycin	Pluramycin
Gancidin	Actinoflocin
Ractinomycin	Griseolutein
Chromomycin	Actinoleukin
Mitomycin	
Acidic substances of low molecular weight	
Sarkomycin	6-Diazo-5-oxo-L-norleucine
Azaserine	Carzinophilin
High molecular weight substances	
Carcinomycin	Carzinocidin
Purine antimetabolite	
Puromycin	
Antifungal substances	
Toyocamycin	Hygroscopin

TABLE 4

Results of screening of actinomycetes for antitumor substances (Umezawa, 1956)

	Ehrlich carcinoma, ascitic form	Mouse sarcoma 180, ascitic form
No. actinomycetes tested	705	98
No. strains producing antitumor substances in first test	130	17
No. strains positive in repeated test	69	

TABLE 5

Results of screening actinomycetes for antitumor substances, using HeLa cells (Umezawa, 1956)

	Number	Toxic to chick embryo fibroblast	Nontoxic to chick embryo fibroblast
Strains tested	522		
Active strains	109		
Strains active in repeated tests	68		
Active against Ehrlich carcinoma, ascitic form	14	9	5
Toxic to mice	19	14	5
No effect on Ehrlich carcinoma, ascitic form	35	20	15

The screening methods devised to isolate antibiotics have been modified for the isolation of antitumor agents (Tables 4 and 5). One such method consists of isolating actino-

mycetes from soil, growing them in liquid media, and testing the filtrates for activity against experimental tumors in mice. The test systems can be solid tumors, ascitic forms, or blood tumors. The results can be evaluated in terms of reduction of tumor sizes, weights of animals, number of tumor cells in the ascitic fluid, or even the prolongation of the life of the treated animals in comparison with the respective conditions of the untreated controls.

To save time and animals, efforts have been made to elaborate preliminary screening programs *in vitro*. Various lines of reasoning have been followed:

1. Cancer cells are rapidly dividing cells. In the search for antimitotic agents, the inhibition of mitosis in onion root tips has been investigated. However, this method gives no information about the selective action of the antimitotic agents found.

2. Cancer cells have active dehydrogenase systems. One can put such cells *in vitro* in presence of an oxidation-reduction indicator such as methylene blue. If the cells are killed *in vitro* they will be unable to reduce methylene blue to the leuco form and they will be stained blue. Here again, the method is not selective, but it can be most useful for assay purposes.

3. Cancer cells have an impaired respiration mechanism. One can thus select for further studies substances which would have a selective antifermentative action or substances that would be selectively active against microbial mutants which would also have an impaired respiration. These microbial mutants can be considered as the equivalent of cancer cells in the microbial work. Good correlation has been shown between antitumor activity and activity against such mutants. This subject was reviewed by Gause in 1959 (Udintzev *et al.*, 1959). Specific studies on different actinomycins were reported by Hackmann and Schmidt-Kastner (1957).

The screening procedure involving tests for antitumor activity can be carried out by the cylinder plate method with carcinoma cells and a dye such as 2,6-dichlorophenolindophenol. This method showed, for example, that Ehrlich carcinoma cells were about 10 times more sensitive to sarkomycin than were cells of Yoshida sarcoma, upon which sarkomycin was almost inactive. Testing of the toxicity on HeLa cells in tissue culture was also found to be useful for the screening and extraction of the active agent. There were substances which were destructive to HeLa cells but not to Ehrlich carcinoma and chicken embryo cells, as shown in Tables 3 to 5 (Umezawa, 1956).

Perlman *et al.* (1959) made a study of effects of antibiotics on multiplication of L cells of mouse fibroblasts in suspension culture. They reported positive inhibition of multiplication with less than 1 μg per ml of actinomycins B, B_V, or D_{IV}; of cycloheximide, gliotoxin, hygromycin, and mitomycin C; of clavacin, thiostrepton, and xanthomycin. Actinomycin B_V was most active, with 1.5 μg per ml causing a 50 per cent reduction in growth of culture. It was suggested that this method can be used in determining the presence of cytotoxic microbial products.

Once an organism with desirable properties, as shown by the screening test used, has been isolated, a series of investigations must be initiated to evaluate the true potentialities of the antitumor substance. Only a few compounds have found practical application. The others either were never described or were described purely because of their academic interest. Some compounds, such as mitomycin, offered great promise but were found to be of little practical value because of their high toxicity. Others, like actinomycin, seemed to be stillborn but were only ahead of their time. Still others, like puromycin, sarkomycin, carzinophilin, azaserine, and DON, proved to be of limited value.

In discussing the problem of anticancer

agents, Chain (1958) made the following comment: "In the absence of any line of rational approach to the problem of tumour therapy, any new substance with any sort of biological activity is tested by someone with regard to its possible inhibitory effect on tumours; there is no *prima facie* scientific case at all why antibiotics in particular could be expected to act against tumours. Bacterial infections and malignant tumours have nothing in common except that in some very superficial resemblance phenomena of growth are involved in both diseases."

Antiviral and Antiphage Surveys

In a recent review of antibiotics, Abraham (1959), one of the pioneers in the field, stated: "In some laboratories they [screening programs] also aimed at the discovery of substances that will selectively inhibit the growth of tumor cells or viruses, though there is so far little evidence to indicate that they will have much success in this field."

In a summary of an early survey dealing with the problem of antiviral agents, Jones and associates (1945) reported that they tested 150 organisms, comprising bacteria, fungi, and actinomycetes, isolated from composts, manures, soils, drainage material, as well as from soil enriched with virus concentrates, for their ability to inactivate viruses. Only three of these organisms gave any indication of possible inactivation of fowl pox virus, and in one case, of the laryngotracheitis virus. An active principle was isolated from a culture of one of these organisms; the substance proved to be actinomycin. The other two virus-inactivating organisms were less extensively studied.

A number of substances capable of modifying or controlling viral infections have been described in recent years. A beneficial effect was found to be exerted on previously infected hosts, such as chick embryos. These compounds included certain bacterial polysaccharides, hexamidine, pectin, tannic acid, tea extracts, crude penicillin, and viscosin. A preparation obtained from a strain of *Streptomyces lavendulae* and designated as ehrlichin also had an injurious effect upon influenza virus (Groupé *et al.*, 1951).

Unfortunately, none of the above observations, or others of a similar nature, led to any practical developments as potential therapeutic agents in viral diseases. Actinomycin received no further consideration because of its highly toxic effect upon animals. Different investigators, constrained by the difficulties involved in the use of animal and plant viruses for this type of investigation, devoted their attention to the bacterial viruses or bacteriophages (Schatz and Jones, 1947; Asheshov *et al.*, 1949, 1952; Hamada, 1957; Smejkal, 1960), on the assumption that if one were successful in obtaining antiphage agents, he would be dealing with mechanisms similar to those that might be destructive to the ultramicroscopic intracellular agents affecting higher forms of life. Several preparations were actually found to be effective against phages, but they were totally inactive for animal viruses.

In an effort to find drugs that would possess antiviral properties, Dickinson (1953) suggested the following possibilities: (1) direct inactivation of the extracellular virus; (2) prevention of adsorption of virus on the cell or of its penetration into the cell; (3) inhibition of multiplication of intracellular virus; (4) prevention of release of the virus from infected host cells.

Hermann and Rosselet (1960) proposed a procedure by which paper chromatograms of antiviral agents are developed by applying them to virus-infected, agar-overlay, chick embryo cell tissue cultures. The plaque-free zones produced indicate the location of the active material on the paper chromatogram. The biological activity of several antiviral agents could thus be demonstrated. As little as 1 μg of an antibiotic (W 122) produced large plaque-free zones when a paper chromatogram was ap-

plied to the overlay culture 1 hour after virus infection; large plaque-free areas resulted when 100 μg were applied 46 hours after virus infection.

Certain glycoproteins in the sap of various plants were found capable of reducing the infectivity of several plant viruses. On dilution, however, noninfective mixtures regained infectivity, thus indicating a lack of combining ratio between virus and inhibitor necessary to cause a loss of infectivity (Kassanis and Kleczkowski, 1948). It has been suggested, however, that the evidence points not to a combination effect of virus and proteins, but rather to the effect of the latter upon the host, or to an antagonistic behavior between the virus and inhibitor. Among the inhibiting factors, the action of ribonuclease on ribonucleic acid viruses is of particular interest. This enzyme hydrolyzes the nucleic acid derived from these viruses, but seems to have no effect upon the intact viruses. When mixed with the virus, however, the enzyme combines with it and inhibits its infectivity reversibly in a manner similar to protein (Bawden and Pirie, 1957). Other substances capable of inhibiting the infectivity of plant viruses include certain yeast polysaccharides (Kleczkowski, 1946).

TABLE 6

Effect of a nocardia antibiotic on tobacco mosaic virus multiplication under different light intensities (Schlegel and Rawlins, 1954)

Preparation	Light	Concentration of antibiotic	Percentage inhibition of virus multiplication	Average per cent deviation from mean
	foot candles	*mg/100 ml*		
Unpurified antibiotic	300	1	69	0.8
	300	1	73	5.5
	25	1	90	7.7
		%		
Purified antibiotic	1100	0.001	73	8.3
	0	0.001	74	4.5
	1100	0.005	86	1.9
	1100	0.001	80	6.3
	1100	0.0001	23	5.9

Some of these substances, like primycin, exert not only antiviral but also antibacterial effects; some were found able to cause marked destruction of worms. These substances vary in solubility, stability, activity, and toxicity.

Schlegel and Rawlins (1954) isolated from a *Nocardia* a substance found to be effective in inhibiting multiplication of tobacco mosaic virus in floating leaf discs. Its action appeared to be relatively independent of changes in host composition produced by light. It was concluded that it inhibits virus increase by acting directly upon virus multiplication rather than on host metabolism (Table 6). Other antiviral agents produced by *Nocardia* have been studied by Harris and Woodruff (1953). Kuroya *et al.* (1957) examined 418 culture filtrates of freshly isolated streptomycetes for their activity against influenza virus cultivated *in vitro*. Of the filtrates 21.5 per cent showed some activity. There was no correlation between activity and any other biological properties of the organism.

Unfortunately, none of these numerous preparations can be said to have a sufficiently suppressive effect upon viral diseases to warrant use in chemotherapy of such diseases. In analyzing the failure to obtain antiviral substances of practical significance, Waksman (1960) concluded that since viruses do not grow and do not metabolize, the whole approach to their suppression must be different from that of antibiotics which act upon the living systems of bacteria, fungi, and other microorganisms.

Further observations on antiviral agents and reasons for failure to obtain good preparations have been examined by Levaditi (1952), Kuroya *et al.* (1957), Krassilnikov *et al.* (1960), and Waksman (1960).

Antitrichomonal Agents

Yamaguchi and Saburi (1955) found that of 1244 cultures of actinomycetes, 172 possessed antitrichomonal properties; a few of

TABLE 7

Inhibition of different actinomycetes by their respective antibiotics (Waksman *et al.*, 1946)

Antibiotic	Organism producing antibiotic	Activity of preparation, per mg	Inhibition of		
			S. antibioticus	*S. lavendulae*	*S. griseus*
			mg/ml		
Actinomycin	*S. antibioticus*	100,000*	10	0.2	10
Streptothricin	*S. lavendulae*	100†	1	2500	100
Streptomycin	*S. griseus*	125†	1	10	830

* *S. lutea* units; crystalline material.

† *E. coli* units; crude preparation.

these lost their activity on repeated transfers in synthetic media. Among the substances so far isolated that possess antitrichomonal properties it is sufficient to mention borrelidin and anisomycin.

Use of Antibiotics for Isolation of Specific Antibiotic-producers

The resistance of different organisms to their own antibiotics and to closely related forms can be utilized for isolating new strains of organisms producing specific antibiotics and for obtaining even more potent strains of such organisms. This phenomenon has long been recognized (Table 7). Waksman, Reilly, and Johnstone (1946) were the first to suggest utilization of this property for the isolation of organisms producing specific antibiotics. This is further illustrated in Table 8.

In a study of a number of strains belonging to the same species, or at least the same series, of *S. lavendulae*, Okami demonstrated (Table 9) the high selectivity in the antagonistic effects of one upon another. The type culture of this species (No. 3330), isolated in 1915, exerted no antibiotic action upon any of the other strains; it was sensitive, however, to three other cultures belonging to this group. Another culture, No. 3555, likewise produced no antibiotic but was sensitive to several other members of the group. The original streptothricin-producing culture, No. 3440-8, was active

TABLE 8

Inhibition of S. fradiae 3554 by different antibiotics (Teillon, 1952)

Antibiotic used (impregnated disc)	Inhibition zone
	mm
Streptomycin, 0.5%	14
Streptothricin, 1.0%	0
Neomycin, 0.25%	0
Oxytetracycline, 0.5%	16
Chlortetracycline, 0.25%	17
Chloramphenicol*	17

*Water saturated solution.

TABLE 9

Reciprocal antagonism between various S. lavendulae strains (Okami*)

S. lavendulae strain streaked	Strain cross-streaked and inhibition zone (mm)											
	3330	3440-8	3516-W	3530	3531	3532	3542	3543	3568	3465	3544	3555
3330	0	0	0	0	0	0	0	0	0	0	0	0
3440-8	8	0	0	0	0	8	0	8	3	8	3	16
3516-W	0	16	0	0	0	12	16	0	18	13	11	13
3530	0	16	8	0	14	12	16	4	12	14	11	14
3531	0	0	12	0	0	4	11	9	10	10	11	1
3532	0	0	0	0	0	0	0	1	2	0	0	0
3542	6	6	7	7	5	7	0	6	6	7	7	18
3543	0	0	0	0	0	0	0	0	0	0	0	0
3568	0	0	0	0	0	0	0	0	0	0	0	0
3465	5	0	0	0	0	0	0	0	0	0	0	0
3544	18	9	14	10	9	17	9	10	13	13	0	6
3555	0	0	0	0	0	0	0	0	0	0	0	0

* Unpublished.

against several strains of *S. lavendulae*, but inactive against others. This was true of some other forms. In no case was one strain autoinhibited.

This should not be considered, however, as justifying the broad generalization, much emphasized by Krassilnikov (1950, 1951, 1958), that because some antibiotic-producing organisms are resistant to their own antibiotics the general conclusion may be reached that antibiotics form a kind of defense mechanism for different microorganisms living in mixed populations. It must rather be looked upon as an isolated instance in a complex group of relationships among organisms living in mixed populations (Waksman, 1956; see also Okami *et al.*, 1960).

Musilek (1957), for example, demonstrated that the growth of *S. aureofaciens* on an agar medium was not inhibited by the antibiotics of the tetracycline group, but was inhibited by other antibiotics. This was true also of an actinomycin-producing actinomycete that was not inhibited by its own antibiotic. On the other hand, *S. griseus*, *S. rimosus*, and erythromycin-producing actinomycetes were not inhibited by the antibiotics produced by these organisms or by similar antibiotics; at the same time, they were not inhibited by various antibiotics produced by different other organisms. The conclusion was reached that the finding of resistance of an unknown actinomycete to a certain antibiotic cannot in itself serve as a method for identifying actinomycetes producing known antibiotics.

Other Antimicrobial Systems

Certain actinomycetes were found capable of producing substances that possess marked bacteriolytic properties. These were quite distinct from phages, although some of them exerted an autolytic effect; they were known long before the true nature of the antibiotics became recognized. This is true of the "bacteriolytic enzyme of Streptothrix" studied by Gratia (Gratia and Dath, 1924–1927) and designated by Welsch (1937) as actinomycetin. It is also true of the "actinomyces lysozyme," recognized by Kriss (1939) and by Krassilnikov and Koreniako (1940). Welsch *et al.* (1955) later isolated from the actinomycetin preparation several substances, one of which was designated as actinozyme; another was designated as actinolysin, and was active against both dead and living bacterial cells. Whether these substances should be considered as enzyme systems, and more specifically, autolytic or generally lytic enzymes, or whether they should be considered as true antibiotics is a subject for further study. Here one approaches the borderline of biological systems that may be considered either antibiotic or enzyme, or even vitamin, since some of the antibiotics have, in certain concentrations, a definite growth-promoting effect upon lower and higher forms of life.

Numerous other preparations possessing antibiotic properties have been isolated from cultures of actinomycetes. These range from well defined compounds such as the phenazines (Yagishita, 1960) to crude preparations. In many cases the information is so limited that it is difficult to place the preparation in one or another of the above groups. Further study of many of these preparations may have been abandoned, since no practical significance could be attached to them. These substances are listed in Part B of this volume.

Chapter 4

Production of Antibiotics

The problem of the possible formation of antibiotics in nature has already been discussed in Chapter 1. In the present chapter the methods used for the empirical production of antibiotics by actinomycetes *in vitro* are discussed. What is known of the biogenesis and the mechanism of biosynthesis of antibiotics by these organisms will be considered in Chapter 7.

The ability to form antibiotics is one of the numerous reactions characteristic of various biological systems. The microbiologist has learned to recognize and favor such systems. The chemist has developed methods for the isolation of antibiotics. Techniques for their rapid production on a large scale have been developed by the engineer. The pharmacologist and the clinician have suggested procedures for their practical evaluation. By combined effort, these investigators have succeeded in obtaining powerful drugs for combatting a variety of infectious diseases. To appreciate the problems involved, one must consider the following facts:

1. Not all kinds of microbes and not even all strains of a particular group of microbes are able to form antibiotics. This phenomenon is not so widely distributed among the microbes inhabiting our planet as to warrant any generalization in regard to their role in microbial life or in the survival of the organisms capable of forming such antibiotics.

2. Different strains of a microbe capable of producing a given antibiotic vary greatly, both qualitatively and quantitatively, in this capacity. Production of an antibiotic is not a fixed property of the organism and is evidenced only when the organism is grown in special media and in selected environments.

3. The ability of a particular microbe to form antibiotics may easily be lost under certain conditions of culture or as a result of mutation.

4. The concentration of a particular antibiotic produced by a given microbe can be greatly increased by strain selection, by development of special media, and by improvement of conditions of cultivation.

5. The same organism may be able to form different antibiotics in different media and under different conditions of cultivation. Each antibiotic is characterized by a given chemical structure and by its antibiotic spectrum.

6. The same culture may have the capacity of forming, in the same medium and at the same time, several antibiotics or various chemical modifications of the same antibiotic. As many as five to seven different, although chemically related, antibiotics have been found in the same culture of the same organism.

7. The same antibiotic, or closely related chemical modifications, can be formed by different organisms.

Among all groups of microorganisms, the actinomycetes have proved to be the richest source of antibiotics. Most of these antibiotics are produced by members of the genus *Streptomyces*; only a few of them are produced by strains of *Nocardia* and *Micromonospora*.

Production of the Same Antibiotic by Different Species

In view of the fact that antibiotic-producing organisms are biological systems, one may expect considerable variation, not only of a quantitative but also of a qualitative nature, among the species and strains producing the same type of antibiotic. Once a given antibiotic has been recognized, it is a common practice to try to increase the yield by strain selection, improvement in composition of media, and changes in environmental conditions such as aeration and temperature.

The production of the same antibiotic or closely related forms by different organisms is recognized for such therapeutically important compounds as streptomycin, tetracycline, neomycin, actinomycin, and various others, notably the polyenes.

The ability of species other than *S. griseus* to produce streptomycin was first demonstrated by Johnstone and Waksman (1948). It has since been established that streptomycin and the streptomycin group of antibiotics are produced by a variety of organisms found among the different series of the genus *Streptomyces* (Volume II, Chapter 6). These include (Okami *et al.*, 1959): (1) tuft-producing organisms with straight aerial mycelium: *S. griseus*, *S. bikiniensis*, *S. rameus*; (2) spiral-producing organisms: *S. humidus*; (3) verticil-producing organisms: *S. reticuli*, *S. griseocarneus*, *S. mashuensis*. *S. griseus* produces streptomycin and mannosidostreptomycin; *S. humidus* forms dihydrostreptomycin (Kavanagh *et al.*, 1960); and *S. griseocarneus* forms hydroxystreptomycin.

Table 10

Classification of actinomycin-producing organisms

(Corbaz *et al.*, 1957)

Organism	Chromogenicity	Actinomycin type*
S. antibioticus	+	X
S. michiganensis	+	X
S. parvullus	−	I, X
S. chrysomallus	−	C, I
S. parvus	−	X

* Brockmann system of nomenclature used.

Neomycin and the neomycin group of antibiotics are produced by numerous strains of *S. fradiae*, *S. roseoflavus*, *S. albogriseolus*, *S. kanamyceticus*, and by a variety of other organisms (Waksman *et al.*, 1958). This group comprises neomycins B and C, neamine, catenulin, kanamycin, paromomycin, and a number of other compounds, the exact chemical nature of which is still undetermined.

The tetracyclines comprise a variety of compounds, including chlortetracycline, oxytetracycline, tetracycline, demethylchlortetracycline, and others. These are produced by different organisms, including *S. aureofaciens*, *S. rimosus*, certain members of the *S. flavus* group, and others.

A number of organisms are now known to be capable of producing actinomycins; they include *S. antibioticus*, *S. flavus*, *S. flaveolus*, *S. parvus*, *S. chrysomallus*, *S. parvullus*, and *S. cellulosae*. Some of the polyenes are produced by a large number of species and varieties (Yajima, 1955). Blinov (1958) demonstrated that the candicidin-type polyene is produced by at least 11 different organisms.

Further information on the chemical relationships of the various groups of antibiotics is given in Chapter 6.

A very interesting relationship between microbial specificity and chemical nature of antibiotics produced is found among the actinomycins (Waksman, Geiger, and Reynolds, 1946). Corbaz *et al.* (1957) demonstrated (Table 10) that although different species of *Streptomyces* are capable of producing actinomycin, the chemical composition of the antibiotic differs. In addition to these organisms, other actinomycetes as well are capable of producing actinomycin, as shown for *Micromonospora* sp. (Fisher *et al.*, 1951).

Production of Different Antibiotics by the Same Organism

Not only may the antibiotic produced by an organism vary in chemical composition, but its very nature may differ (Trussel *et al.*, 1947). This can easily be demonstrated by the fact that frequently the same organism may produce both antibacterial and antifungal antibiotics. Thus, *S. griseus* was found to produce streptomycin, candicidin, and cycloheximide; *S. fradiae* produces neomycin and fradicin; *S. rimosus* forms oxytetracycline and rimocidin. Frequently, different strains of the same organism produce different antibiotics. This is true, for example, of different strains of *S. lavendulae*, which produce several forms of streptothricin and also a variety of such compounds as ehrlichin and polyenes. Often a change in composition of medium and in conditions of growth results in a change in the nature of the antibiotics produced by the same organism. Certain organisms are capable of forming in the same medium as many as three different antibiotics (Despois *et al.*, 1956). *S. albireticuli*, for example, produces eurocidin, enteromycin, and carbomycin (Osato *et al.*, 1955).

Production of Antibiotics in Liquid and Solid Media

As was shown in Chapter 3, the fact that an actinomycete has antagonistic properties sometimes may not be discovered until after it has been grown both on solid media and in liquid media. For instance, the culture may form zones of inhibition against test organisms on solid media, but may refuse stubbornly to produce antibiotics in liquid media. This may be due to differences between the cultural conditions of some actinomycetes on the two different types of media. In certain cases, for example, this phenomenon can be explained by the formation of ammonia by the actinomycete. It can easily be demonstrated that some actinomycetes can produce a volatile substance which is toxic to fungi. Inhibition of the fungi can be noted not only on the layer of agar on which the organism is growing, but also on a layer that is on top of it and separated by an air space. The pH of this upper layer of agar increases during the growth of the actinomycete on the layer below, and fungi seeded on this upper layer will not grow, whereas some bacteria will. Substitution of a dilute solution of ammonia for the growing actinomycete produces a similar inhibitory effect on fungi. These experiments do not rule out the possibility that actinomycetes form volatile antibiotics different from ammonia, but the production of this compound can probably explain certain discrepancies between inhibitory effects observed when actinomycetes are grown on solid and in liquid media.

In general, once an antagonistic actinomycete has been selected in a screening program, the antibiotic or antibiotics produced by this organism must be obtained in large enough quantities to permit their extraction, purification, characterization, and if necessary, the study of their toxicity, pharmacology, and activity *in vivo*. This is accomplished by growing the antagonist in liquid cultures which are incubated on shaking machines or in fermentors of various volumes.

The motion applied to flasks by shaking machines is either circular (rotary shakers) or linear (reciprocal shakers). The purpose of the shaking is to furnish the culture with the aeration necessary for maximal growth and the formation of antibiotics. The motion may also play a role in keeping the organism well dispersed in the liquid medium, permitting good contact between the cell surfaces and the nutrients.

Assay Methods

Since antibiotics are chemical substances capable of inhibiting the growth of micro-

organisms, they can be assayed chemically and/or biologically (Grove and Randall. 1955).

Chemical Methods

Chemical methods can be used only after the chemical properties of a given antibiotic are known. Examples of these chemical assays are: (1) use of the blue-colored ninhydrin reaction to assay antibiotics with free amino groups, such as neomycin; (2) the use of maltol determination to assay streptomycin and mannosidostreptomycin; and (3) the anthrone test to titer mannosidostreptomycin by determining the amount of mannose present. Stable antibiotics with strong and typical light-absorption spectra can be assayed directly by spectrophotometric methods. Chemical methods of assay must be used with caution, since contaminating substances may give false positive values. For example, contaminating amino acids would give a positive ninhydrin reaction and interfere with the chemical assay of neomycin by this reaction.

Biological Methods

Biological methods of assay of antibacterial and antifungal antibiotics are of two general types: (1) dilution and (2) diffusion methods. Dilution assays can be made with or without a standard. It is virtually impossible to make diffusion assays without a standard.

Dilution assays can be made in both liquid and solid media. The use of solid media permits the assay of a given antibiotic against a number of test organisms with minimal labor. The dilution assay on solid media, one of which was described by Waksman and Reilly (1945), is a good procedure for use without a standard. Activity is expressed in terms of the minimal amount of antibiotic that will prevent growth of a test organism. The measure can be expressed in micrograms per milliliter or in dilution units.

Dilution assays in liquid media are well suited to statistical analysis; a standard is usually required. In such an assay, the turbidity caused by the growth of a bacterium or a yeast is measured and the value of unknown preparations is determined by estimating the amount of unknown necessary to permit the same growth as that permitted by a known amount of the standard.

Factors influencing the results of dilution assays are: (1) the composition of the medium (especially its pH and salt content), and (2) the age and number of cells of the test organisms used to inoculate the medium.

Diffusion assays are carried out by placing cylinders or filter paper discs containing various amounts of an antibiotic solution on the surface of an agar layer seeded with a test organism. Comparison of the sizes of sterile zones produced by solutions of unknown concentration with those produced by known solutions of a standard permits estimation of the potency of unknown preparations.

Factors influencing diffusion assays are: (1) the thickness and evenness of the agar layer, (2) the rate of evaporation of water from the agar layer, (3) the composition of the agar medium (especially its pH and salt content), (4) the nature of the solvent containing the antibiotic samples (especially pH and salt content), and (5) the method of seeding the agar layer with the test organism, sharper zones of inhibition being formed if a thin agar seed layer is superimposed on a thicker unseeded layer of agar.

A comparative study was recently made of the relationship between the minimal inhibiting concentration of an antibiotic by the serial dilution method and the diameter of the zone of inhibition by the single-disc method. It was found to correspond to a straight line, with the exception of spiramycin for which the values fit a second order equation (Ericsson *et al.*, 1960). The measurement of antibiotic concentrations in

clinical medicine must take into consideration the phenomena of absorption and distribution in different body fluids (Ericsson, 1960).

Increasing Commercial Yields

For the chemical characterization of an antjbiotic, broths of low titer of activity may be satisfactory. However, for its commercial production, broths of the highest possible titers are desired. Yields can be increased by two general approaches, which are usually utilized together. One can either (1) improve the strain forming the antibiotic by selection of more and more active mutants, or (2) improve the media and cultural conditions used for the growth of the antibiotic-producing organism.

Strain Selection

The ability of different strains of the same organism to form different concentrations of the same antibiotic is illustrated in Table 11.

TABLE 11

Antibiotic potency of different strains of S. fradiae, group I (Waksman *et al.*, 1958)

Medium: N-Z-amine-glucose. Potency expressed in neomycin units per ml of broth.

Strain No.	Incubation period		
	3 days	4 days	6 days
3535	270	285	324
3554	325	230	195
3556a	202	241	375
3556b	279	239	297
3572	67	77	78
3594	394	321	687
3719	200	264	230

At least four distinct procedures are utilized for obtaining more active strains capable of producing higher concentrations of a particular antibiotic:

1. Selections from a natural population of a culture and evaluation of antibiotic potency of different strains of the particular culture.

2. Selection of strains from a given culture grown on a medium enriched with the antibiotic produced by the particular culture.

3. Preliminary treatment of a culture with ultraviolet and other radiations.

4. Treatment of a culture with various chemicals, such as ethylene amine, nitrogen mustard, or colchicine.

Natural selection is accomplished by plating out actinomycetes so as to get a large number of isolated colonies, each one originating, if possible, from a single spore or from small segments of mycelium. The level of antibiotic production of these substrains can be tested in a number of ways. The substrains can be tested directly in liquid or in solid media. In this case replication methods are often used as a time-saving device, but in final analysis an increase in antibiotic formation in liquid media is the aim.

Actinomycetes, as a rule, are sensitive to strictly antibacterial antibiotics and resistant to strictly antifungal antibiotics. These properties can be utilized to isolate strains of actinomycetes which produce antibacterial antibiotics of a given type. Active strains will be more resistant to the action of the antibacterial antibiotics they produce than will other actinomycetes, and the more antibiotic a given strain will accumulate, the more resistant the strain will be. That this phenomenon is not universal, however, is evident from the fact that strains of *Streptomyces rimosus* forming oxytetracycline are resistant to streptomycin.

Waksman *et al.* (1946) first suggested the use of a streptomycin-enriched medium for the selection of streptomycin-producing cultures. Although the inactive variants were thus eliminated, more active cultures were not obtained. This method was later utilized by McDaniel and Hodges (1950) with considerable success and resulted in the produc-

tion of highly active cultures. When this method is combined with other procedures, such as radiation treatments, highly active substrains can be obtained. Strain selection of streptomycin-producing organisms has been studied further by Savage (1949), Dulaney *et al.* (1949), Dulaney (1953), and Pittenger and McCoy (1953).

Exposure of antagonistic actinomycetes to various mutagenic agents has been the most successful method of obtaining better antibiotic-forming strains. In this area, ultraviolet light has played an important role, as shown by the following procedure of Darken *et al.* (1960), who discussed the methods used to improve tetracycline-forming organisms. An isolate was selected from among the better producers. It gave an average of 125 μg per ml of antibiotic. A spore suspension of the culture was irradiated with ultraviolet light at an exposure sufficient to kill 99.99 per cent of the spores, and 200 colonies were transplanted to agar tubes. A total of 19 isolates, or 9.5 per cent, showed various types of gross morphological variation. When X-ray treatment was used, an exposure of 100,000 roentgen units gave 99.5 per cent kill. A total of 340 colonies was picked and, of these, 149 (44 per cent) were gross morphological variants. Of 45 isolates obtained by natural selection and 135 by X-ray treatment, none showed a significant increase in antibiotic production. Treatment with ultraviolet irradiation, however, resulted in three superior isolates.

A culture giving a 20 per cent higher tetracycline yield than the original isolate, or an average of 150 μg per ml, was selected for medium development studies; 1250 derivatives of this culture were examined. Of these, 160 (23 per cent) of 700 ultraviolet-treated isolates and 190 (38 per cent) of 500 X-ray-treated isolates were morphological variants. None of the 50 natural selections, however, gave evidence of gross morphological change (see also Katagiri, 1954). The variability of naturally occurring oxytetracycline-producing strains has been studied by Borenstain and Wolf (1956).

There was a greater number of superior antibiotic-producers as well as a higher incidence of morphological types in the X-ray-treated isolates than in either the ultraviolet-treated or the untreated isolates. Of 640 ultraviolet-treated cultures, only three gave significant increases in broth potency over the parent. Of these, a culture was selected as a control for the testing of 390 derivatives from the X-ray treatment of one culture (UV8). The yields obtained with these selected isolates from this culture grown in a synthetic medium ranged from an average of 1955 to 2150 μg per ml.

Goldat (1958) reported that the treatment of spores of *S. aureofaciens* with various doses of ultraviolet rays causes an increase in the mutation rate. A high dose of ultraviolet rays and a four-fold photoreactivation gave the largest number of morphological mutations; an exposure to smaller doses was found to be more effective for increasing the variation of activity. A single photoreactivation with daylight reduced both the lethal and the mutational effects, whereas a repeated reactivation increased the latter.

Alikhanian (1959b) discussed the results obtained by various investigators in improving strains of *S. griseus*, *S. rimosus*, and *S. aureofaciens* in order to increase production of streptomycin, oxytetracycline, and chlortetracycline. Table 12 shows the predominant role played by ultraviolet light and X-rays.

Some chemical mutagenic agents such as nitrogen mustard have also found a place in the search for more active mutants. Among other mutagenic agents, actinophage may be mentioned (Ilina and Alikhanian, 1957). Further studies on the genetic interactions among streptomycetes and the selection of active strains is found in the work of Bradley

TABLE 12

Strain selection by various methods of treatment (Alikhanian, 1959b)

Organism and antibiotic	Year	Author	Treatment	Activity	
				Old strain	New strain
				units/ml	
S. griseus-streptomycin	1947	Stanley	Ultraviolet rays	400	900
	1949	Savage	Ultraviolet rays and X-ray	180	700
	1953	Pittenger and McCoy	Ultraviolet rays	180	800–900
	1949	Dulaney *et al.*	Ultraviolet rays	250	1000
	1953	Dulaney	X-ray	1000	2000
	1957, 1959	Alikhanian	Ultraviolet rays and visible light	3200	4000
S. rimosus-oxytetracycline	1956	Borenstain and Wolf	Ultraviolet rays and nitrogen mustard gas	500	1500
	1958	Mindlin and Alikhanian	Ultraviolet rays	1828	3040
S. aureofaciens-chlortetracycline	1954	Katagiri	Ultraviolet rays and nitrogen mustard gas		1200–1300
	1958	Goldat	Ultraviolet rays	600	1000
	1959	Goldat	X-ray	1000	2200
	1959	Lin Wang	X-ray	400	1000

et al. (1959), Alikhanian *et al.* (1959), and Mindlin and Alikhanian (1958).

Improvement of Media and Cultural Conditions

During the development of a process for the production of a given antibiotic, the work on strain selection and the study of media and cultural conditions go hand in hand.

In improving media, the following nutrients must be investigated: (1) sources of carbon, (2) sources of nitrogen, (3) mineral sources, and (4) growth-promoting substances.

Several important factors are known to influence the growth of the antibiotic-producing organism: (1) temperature, (2) initial pH and control of pH during growth, (3) aeration, and (4) agitation.

The medium used to grow the inoculum, the age of the inoculum, and its size will markedly influence the production of antibiotics.

Effect of Composition of Medium on Antibiotic Production

The composition of the medium has a highly important effect upon the growth and metabolic activities of actinomycetes, and upon the production of antibiotics. Both the quantitative yield of specific antibiotics and their chemical structure are greatly influenced by the nature of the nutrients (carbon and nitrogen sources), their concentrations, and the presence of specific salts.

When streptomycin was first produced by freshly isolated strains of *S. griseus* (Schatz, Bugie, and Waksman, 1944), the maximal yield of the antibiotic was 100 to 200 μg per ml. In recent years, this yield has been increased to between 5 and 10 mg per ml. Although the proper strain development had a great deal to do with this phenomenal increase, the selection of suitable media and optimal conditions of growth were also largely responsible.

The addition of basic amino acids (argi-

TABLE 13

Composition of four media found very suitable for production of antibiotics by actinomycetes

(Warren *et al.*, 1955)

Media components	Media numbers and composition			
	A-4	A-4h	A-9	A-12
	gm/liter			
Soybean meal FF grits, edible grade	10.0	15.0		10.0
Peptone, technical grade			5.0	
Corn steep (liquid)				20.0
Glucose, technical grade	10.0	15.0	10.0	
Molasses, Brer Rabbit (green label)			20.0	
Dextrin				10.0
Curbay, B. G.		5.0		
Glycerol		2.5		
NaCl	5.0	5.0		5.0
$CaCO_3$	1.0	1.0		2.0
K_2HPO_4				2.0

nine, histidine, and leucine) to an autoclaved aqueous extract of soybeans increased considerably the yield of streptomycin. The addition of monoamino acids (glutamic, aspartic) was found to be unfavorable for both growth and streptomycin production (Kazanskaia and Andreeva, 1959).

The various constituents of production media are selected on the basis not only of their value in permitting the elaboration of the antibiotics, but also of their costs. Soya bean and corn products have been important in this respect.

Warren and coworkers (1955) showed that the four media listed in Table 13 permitted the production of a large variety of antibiotics by actinomycetes. They concluded that the production of most antibiotics of actinomycetes should occur at a detectable level in at least one of them.

TABLE 14

Influence of nitrogen source on composition of actinomycin complex

(Waksman *et al.*, 1958)

Organism	Nitrogen source	Relative percentage of components						
		I	II	III	IV	V	VI	VII
S. antibioticus	L-Glutamic acid	6.2	2.1	2.8	80.4	8.4		
S. antibioticus	L-Threonine	7.1	2.4	2.9	29.2	58.4		
S. antibioticus	L-Glutamic acid + sarcosine	7.1	24.6	35.6	27.2	5.5		
S. chrysomallus	KNO_3				10.0		50.0	40.0
S. chrysomallus	Glycine				7.0		43.2	49.8

TABLE 15

Influence of amino acids on actinomycin synthesis

(Katz and Goss, 1958)

Medium	Concentration of amino acid added	Relative percentage of actinomycin components					
		I	II	III	IV	V	*
	%						
Glutamic acid	0.0	6.4	2.3	3.2	68.3	17.2	2.6
Glutamic acid + hydroxy-L-proline	0.25	31.0	3.8	7.1	25.3	30.0	3.0
Glutamic acid + sarcosine	0.05	9.1	25.6	35.0	24.4	5.9	0.0
Glutamic acid + N-acetylglycine	0.25	8.1	2.0	3.2	32.7	51.1	3.0
Glutamic acid + L-isoleucine	0.25	4.7	2.8	5.8	17.2	30.6	38.9

* Denotes an unidentified component which moves faster than Component V in circular paper chromatography.

The effect of the medium upon the nature of the antibiotic can best be illustrated by an analysis of the production of different actinomycins. Schmidt-Kastner first demonstrated (1956) that by increasing the amino acid content of the medium or by the addition of special amino acids, notably sarcosine, new actinomycins can be produced. These results were fully confirmed in subsequent studies carried out in our laboratories, as shown in Tables 14 and 15.

An examination of the chemical nature of the actinomycins produced by different organisms revealed certain pertinent differences in the concentrations of the various components of the actinomycin molecule. *S. antibioticus* (the original producer of actinomycin A) gives, in certain media, the following actinomycins: I, 10 per cent; IV, 60 per cent; V, 30 per cent. *Streptomyces* 3723 (the original producer of actinomycin B) gives, in certain media: I, 10 per cent; IV, 30 per cent; V, 60 per cent. *S. chrysomallus* (the original producer of actinomycin C) gives: IV, 10 per cent; V, 50 per cent; VII, 40 per cent. *S. parvullus* (the original producer of actinomycin D) gives: IV, nearly 100 per cent.

Katz and Goss (1958) have demonstrated that it is possible to change to a limited extent the proportion of any particular component in a natural mixture of actinomycins. Certain components normally produced in small amounts may be increased until they represent the major constituents of an actinomycin complex. Actinomycin V, for example, was the major component in the actinomycin mixture produced by *S. antibioticus* when L-threonine was the sole nitrogen source, whereas actinomycin IV represented the main constituent when L-glutamic acid was used. Schmidt-Kastner (1960) demonstrated that actinomycin IV increased from 10 to 83 per cent of the actinomycin mixture formed by *S. chrysomallus* when 0.5 per cent DL-valine was added to a glycerol-nitrate medium.

Various amino acids, notably hydroxy-L-proline, sarcosine, N-acetylglycine, and L-isoleucine, bring about marked quantitative changes in the actinomycin complex produced. Components normally formed in small amounts were found, in certain specific conditions, to represent major constituents of such mixtures.

Among the many other factors influencing the quantitative yield of an antibiotic, the mineral constituents occupy a prominent place. In this connection, the importance of the effect of phosphorus starvation upon the yield of streptomycin must be emphasized. The presence of iron in the medium will favor the accumulation of mannosidostreptomycin, whereas the addition of calcium will lead to the transformation of the latter to streptomycin. Detailed studies of the role of trace elements in the production of streptomycin have been made by Chesters and Rolinson (1951) and numerous others.

Chapter 5

Isolation and Identification of Antibiotics of Actinomycetes

Principles of Isolation

The isolation of antibiotics of actinomycetes requires the use of methods which have found general application in the isolation of natural products.

As will be discussed in detail in Chapter 6, antibiotics of actinomycetes have extremely diverse chemical structures. We find among them acids, bases, amphoteric compounds, neutral compounds, polypeptides, aminosugar complexes, compounds with huge lactone rings, nitro compounds, guanido compounds, polyenic compounds, and acetylenic compounds. One cannot help being amazed by the synthesizing capacities of this group of organisms.

Most antibiotics are released by actinomycetes into the culture medium; a few of them are formed in the mycelium. Clearly then, no single procedure will isolate all these compounds.

When one is dealing with an unknown substance, it is always advisable to have an idea of the stability of the substance at various temperatures and at various pH values before attempting to devise a method of extraction. A reliable assay is also necessary to follow the fate of the antibiotic during those various attempts.

Since antibiotics are rarely formed alone by the producing organism, but are often elaborated as mixtures of complexes of related substances or along with totally unrelated antibiotics, assay methods should give not only quantitative data but also information of a qualitative nature. Qualitative data permit one to follow the fate of the various components of the complexes as they are resolved by the extraction procedures.

Some crude antibiotics are formed of components which have a synergistic action. Without proper bioassays, the activity would seem to disappear during purification. This is true of the antibiotic complexes E 129 and PA 114.

Basically there are two types of methods for separating antibiotics from accompanying impurities: (1) those which take advantage of differences in solubility between compounds and (2) those which take advantage of differences in the affinity of such compounds for the surfaces of adsorbents or ion exchangers. Both methods have endless variations and are often used together in the isolation of a given substance.

The reader will find in Part B of this book outlines of the extraction and purification procedures used for each one of the antibiotics described. He will note that for many antibiotics, various methods of extraction have been used. The quality of the results obtained with a given method will often depend on the medium used for growing the producing organism. Certain nutrients will interfere with a given method of extraction, just as metabolites formed on a given me-

dium may also affect the ease of purification. Thus, it is wise to have close cooperation between workers involved in media development and those who carry out the extraction of an antibiotic.

Methods of Characterization

The characterization of an antibiotic involves the determination of its properties: physical, chemical, and biological. Plants, animals, and other living systems cannot be easily classified on the basis of a single characteristic which would be different for each living form. Similarly, one cannot usually differentiate between the products of living systems on the basis of one single criterion; this is true particularly of the various antibiotics. A number of properties should be studied, and any comparison between antibiotics must be based on a number of characteristics.

It is impossible to make a list of "classical" criteria which would always permit one to differentiate between two antibiotics and which would always be adequate for the full characterization of an antibiotic. A list satisfactory at one time will be inadequate a few years or even a few months later. Scientific methods are forever changing. The investigator must be adaptable and refrain from taking a dogmatic stand.

We will discuss various criteria which have been used in the characterization of antibiotics. In the keys found in Part B of this book we will try to use these characteristics to single out each antibiotic which had been described by the time that the compilation was completed. This will not always be possible, since some substances have not been described in sufficient detail. In such cases we simply group similar substances together.

Nature of Antibiotic-producing Organisms

It has been observed that certain types of cultures tend to form certain types of antibiotics. For example, members of the *Streptomyces griseus* group have been found to produce streptomycin, cycloheximide, grisein, and candicidin, to name only a few. Similarly, strains of *S. fradiae* tend to produce antibiotics of the neomycin type and fradicin. However, the ability of a culture to produce a particular antibiotic is hardly a criterion for the identification of the organism or of the antibiotic. This is due partly to the confused state of the systematics of this group of organisms. Further, it has been shown that the same antibiotic can be produced by a variety of actinomycete species or even by various genera. In this respect the paper of Fisher and coworkers (1951) is of interest. These authors isolated and chemically characterized actinomycin from a strain of *Micromonospora*. This antibiotic is usually produced by streptomycetes and is known to be a mixture of closely related substances. It is then conceivable that the actinomycin elaborated by the *Micromonospora* might have been different from the actinomycins of the streptomycetes. But any difference must have been minor indeed, since the amino acid content of the *Micromonospora* actinomycin was not unusual. It is also unlikely that a *Micromonospora* would be confused with a *Streptomyces*. In this light, one should consider with reserve the statement of Krassilnikov (1960) that authors who claim that the same antibiotic can be produced by different species "are mistaken either in species identification or in the accuracy of determining the nature of the antibiotic."

Teillon (1953) suggested a scheme for the separation of antibiotic-producing organisms on the basis of their sensitivity to known antibiotics (Table 16).

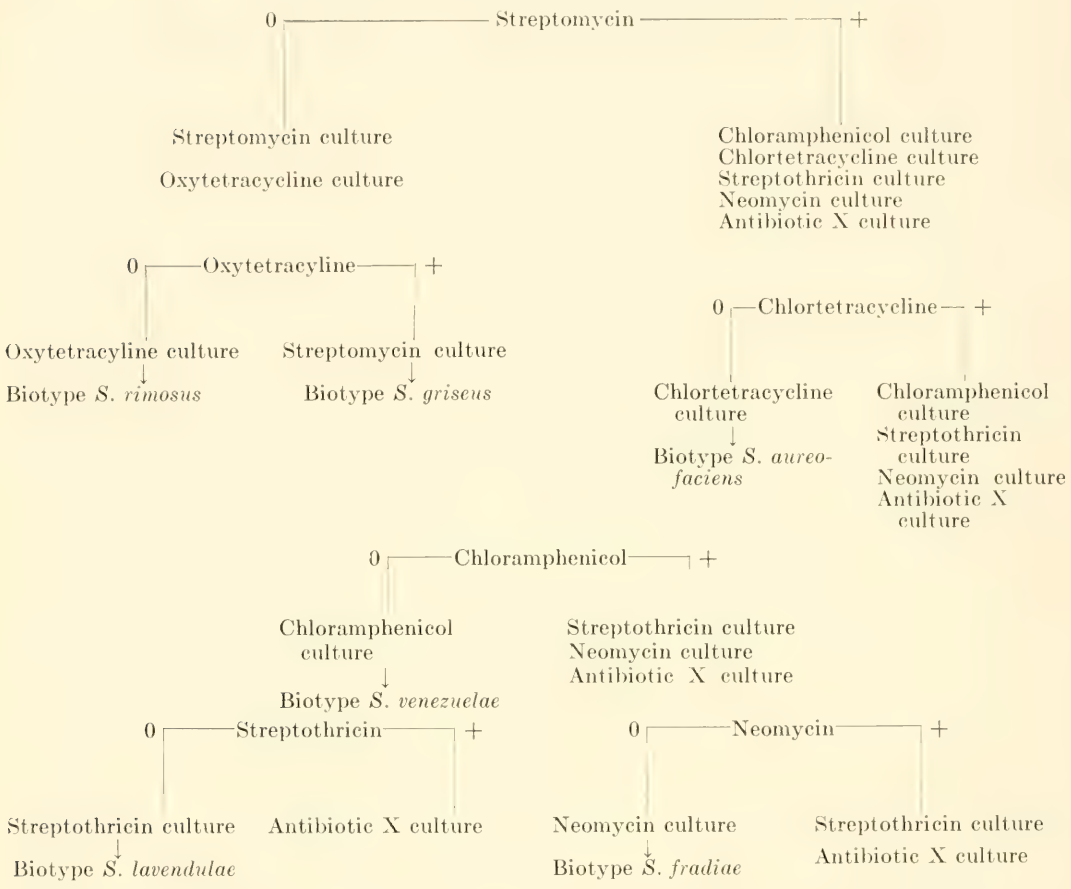
Antimicrobial Spectra

The range of antimicrobial activity of any antibiotic should be determined against aerobic bacteria, both gram-positive and

TABLE 16

Dichotomic key for identification of Streptomyces antibiotics (Teillon, 1953)

+ = positive inhibition; 0 = no inhibition.



gram-negative, anaerobic bacteria, mycobacteria, actinomycetes, and fungi, both filamentous and yeast forms. In such determination of activity, dilution methods are preferable to diffusion methods, since some antibiotics which are very active on a weight basis do not diffuse readily in agar media. If possible, such an antibacterial-antifungal spectrum should be complemented with studies of antiprotozoal, antialgal, antirickettsial, antiviral, and antitumor activity.

Two different antibiotics may have very similar antimicrobial spectra. This is true of the antifungal polyenes candidin and amphotericin B, which have been differentiated biologically only with great care (Lechevalier, 1960).

Mutants of microorganisms resistant to known antibiotics are usually included among the test organisms used for the determination of antimicrobial spectra. If there is no cross-resistance between two otherwise closely related antibiotics it can be assumed that the antibiotics are different. However, there usually is cross-resistance between closely related compounds and this may even occur between very different substances, as was demonstrated by Pledger and Lecheva-

lier (1956), who found cross-resistance between certain antibiotics, such as neomycin, and a mild silver protein.

In the case of streptomycin, dependent mutants are available which will not grow except in the presence of streptomycin or certain of its derivatives.

Development of Resistance

If a large number of cells of a given microbe are subjected to an antibiotic, most of the cells will be inhibited, but a few will grow. These cells will be resistant to either low or high concentrations of the antibiotic. A population of *Escherichia coli*, for example, will contain a few cells that are very resistant to streptomycin. Only a few cells in the particular population of *E. coli* are slightly more resistant to neomycin than the bulk of the cells; the slightly more resistant population can again be put in contact with neomycin and still more resistant mutants will be selected. The ease with which mutants of a given organism resistant to an antibiotic can be obtained is a useful criterion for the characterization of the substance. However, one must keep in mind that development of resistance to one given antibiotic may be fast for one microorganism and slow for another. Polyenic antifungal antibiotics are noteworthy examples of substances to which resistance develops slowly and never achieves high levels. The practical problems in chemotherapy arising from the development of resistance are discussed in Chapter 10.

Microbiostatic and Microbicidal Action

If a microbial population is subjected under identical conditions to concentrations of antibiotics high enough to prevent growth, and if after a period of contact the antibiotics are removed and the cells placed in a suitable nutrient medium, one can easily demonstrate that some antibiotics will have killed the test organism whereas others will simply have prevented its growth. Lechevalier (1960) has shown differences in the fungicidal action of polyenes which could be useful in the characterization of these polyenes.

Use of Inhibitors

The antimicrobial action of certain antibiotics is inhibited by specific substances. For example, the action of streptomycin is inhibited by cysteine, hydroxylamine, and nucleic acids; that of neomycin, only by nucleic acids. The action of chlortetracycline on *E. coli* has been found to be competitively inhibited by riboflavin. The action of oxytetracycline against both gram-positive and gram-negative bacteria is reversed by magnesium ions, whereas in the case of novobiocin, magnesium sulfate reverses the inhibition of gram-negative bacteria but not that of gram-positive bacteria (Brock, 1956).

Mode of Action

Each antibiotic inhibits one metabolic reaction or a series of such reactions in the microbial cell. These reactions can be used as criteria for the characterization of the antibiotic. The mode of action of antibiotics in general is poorly understood (see Chapter 9), but some antibiotics have become biochemical tools for the study of microbial metabolism. For example, chloramphenicol is an inhibitor of protein synthesis; nystatin inhibits endogenous respiration and glycolysis in fungal cells; and antimycin A is an inhibitor of electron transport in the cytochrome C system.

The effect of the antibiotic on the microbial cell may at times alter the morphology of the sensitive organisms. For example, low concentrations of fradicin induce the cells of *Candida albicans* to become filamentous, whereas other antifungal antibiotics, such as nystatin and candicidin, do not induce any change in the form of the yeast.

Physical Properties of Antibiotics

SOLUBILITY

A survey of the solubility of an antibiotic in various organic solvents and in water, at various pH values, is a very useful method of characterization. This method also gives information about changes in the substance being investigated.

An acidic substance will be least soluble in water at an acid pH and a basic substance will be least soluble at an alkaline pH. An amphoteric substance will be least soluble at the pH of its isoelectric point. This information can be supplemented with potentiometric titrations.

One must remember that the solubility of a crude preparation can be very different from that of the pure antibiotic. Thus, crude fradicin is soluble in ethanol, and crystalline fradicin is soluble only in the glycols. As a rule, the purer the substance, the less soluble it is. Candicidin is a good example: crude, it is partly soluble even in water; pure, it is one of the least soluble antibiotics.

STABILITY

As we have already discussed, knowledge of the stability of an antibiotic at various pH values and at various temperatures is an essential prelude to the study of purification procedures. Stable antibiotics such as chloramphenicol and neomycin will not lose activity if autoclaved at a neutral reaction. Other antibiotics, such as mycomycin, are highly unstable. The half-life of mycomycin is only 3 hours at 27°C. In this respect, certain antibiotics are most unusual; for example, upon standing, rifomycin B increases in activity as a result of molecular rearrangement and subsequent transformation into a more potent antibiotic.

COLOR REACTIONS

Chemists have devised a number of color reactions which are characteristic of certain molecular structures, such as the ninhydrin reaction for primary amino groupings, the Sakaguchi reaction for guanido groups, and the Fehling test for reducing sugars. The application of such tests to antibiotics and their degradation products is very useful in characterizing the antibiotic.

In this area, it should be mentioned that certain antibiotics fluoresce when excited by ultraviolet light. The bright yellow fluorescence of oxytetracycline is one example.

LIGHT ABSORPTION

The light absorption of a substance can be measured in the ultraviolet, the visible, and the infrared range. To be of any significance, the infrared spectrum of a substance must be measured on the pure compound. In certain cases, such as that of the neomycins, the infrared spectrum gives little information. In other cases, it will give the chemist considerable information on the various functional groupings which are present in the molecule of the antibiotic. In contrast, certain antibiotics have such characteristic and intense visible or ultraviolet light spectra that they can be detected in the crudest extracts. This is true of the polyenic antifungal antibiotics. If two substances have similar absorption spectra, this indicates the presence in the two molecules of similar groupings but does not mean necessarily that the two substances are identical. For example, trichomycin and candicidin are two different antibiotics with the same light-absorption spectrum.

Chromatography

Chromatography is a method which can be used not only to purify antibiotics, but also to characterize them. In this respect, paper chromatography has been of great value. It can be carried out in either the vertical (ascending and descending) or the horizontal plane (circular and centrifugal).

It can also be carried out more than once on the same sheet of paper (two-dimensional chromatography). Paper chromatography can be used to compare the Rf value of one antibiotic with that of another or to compare the decomposition products or the derivatives of one antibiotic with those of another. The antibiotic can be located on the paper chromatogram biologically (bioautogram) or by chemical color reactions. Laboratories actively engaged in the search for new antibiotics have developed a number of solvent systems which permit separation of most of the known antibiotics into small subgroups. Despite the fact that such paper chromatographic systems play an important role in the characterization of antibiotics, it should not be taken for granted that two substances are identical if they have identical Rf values in a number of solvent systems. Neomycin B and neomycin C, for example, were resolved by paper chromatography only after being acetylated (Pan and Dutcher, 1956).

Electrophoresis

Similar antibiotics may, in certain cases, be separated from one another by submission to a difference in electric potential in a buffered environment. For the isolation and characterization of antibiotics, electrophoresis is carried out either in agar or on paper.

Countercurrent Distribution

Countercurrent distribution is another method that can be used for both purification and characterization of antibiotics. Similar in principle to partition chromatography, this method permits separation of substances as the result of differences in partition coefficients between two immiscible solvent systems. More laborious than chromatography, the method requires costly instruments. Countercurrent distribution is often used as a criterion of purity, especially for antibiotics that cannot be crystallized.

Elementary Analysis

The elementary analysis of a pure preparation of antibiotic is, of course, necessary for the determination of its empirical formula. An elementary analysis of crude preparations of antibiotics can also be most useful for characterization. Its usefulness is limited, however, primarily to the negative results obtained: such analyses can show, for example, that a substance contains no halogens, no nitrogen, or no sulfur.

Physical Constants

Once a new or unknown antibiotic has been obtained in a pure form it can then be characterized on the basis of its physical constants—such as density, refractive index, molecular weight, melting point, and optical rotation—and the physical constants of its salts and derivatives.

Eventually, the substance is fully characterized when its exact chemical structure is known in all its spatial intricacies.

Classification of Antibiotics of Actinomycetes

The antibiotics of actinomycetes represent a great variety of chemical compounds. They vary greatly in their physical properties, including stability to heat and to oxidation, and in their solubility in water and organic solvents. They vary also in their chemical composition, in their antimicrobial activities, and in their toxicity to animals. These differences are frequently not only qualitative but also quantitative in nature. Hence, it is difficult to classify them. However, various systems for the classification of antibiotics have been proposed.

Several criteria have been used in establishing such systems. These are based upon (1) the nature of the organisms that form the antibiotics, (2) the specific antimicrobial spectra or selective action of the antibiotics upon different groups of microorganisms, (3)

the practical utilization of antibiotics in the control of human, animal, or plant diseases, and (4) the chemical nature of the antibiotics. Each of these systems has certain distinct advantages and disadvantages.

BIOLOGICAL SYSTEM

The biological system offers the advantage of a knowledge of the specific nature of the organisms producing the antibiotics. It has even been argued that the ability of a certain organism to form a particular antibiotic is more characteristic of the species than such properties as pigment production, sugar utilization, or enzyme formation. The fact, however, that the same antibiotic may be produced by different organisms and, further, that the same organism, or even strain, may yield more than one antibiotic, reduces the usefulness of this system of classification designed primarily for characterization purposes. The use of this system is further complicated by the great variability of the antibiotic-producing organisms, including ordinary morphological, cultural, and biochemical variations and hereditary mutant formations; the latter may involve either a gain or a loss of the ability to produce a certain antibiotic. Differences in chemical composition or physical state (such as semisolid *versus* liquid) of media used for the cultivation of antibiotic-producing organisms, in the degree of aeration of the culture, and in the length of the incubation period may all result in chemical differences in the antibiotics produced. The sensitivity or resistance of the organisms to their own antibiotics and the ability of certain organisms to decompose the antibiotics after they have been formed are other limiting factors of such a system. Too little is yet known of the mechanism of formation of the antibiotics in the mycelium and their liberation into the medium to permit use of this reaction for classification purposes.

ANTIMICROBIAL ACTIVITIES OR ANTIBIOTIC SPECTRA

The antimicrobial activities or antibiotic spectra offer another system for classifying antibiotics. A knowledge of the range of antimicrobial activities of an unknown antibiotic may permit its ready identification with known substances. Naturally sensitive and resistant organisms, as well as organisms made resistant to known antibiotics, can be used in the classification of antibiotics in general. Unfortunately, different strains of the same organism may vary considerably in their sensitivity to a given antibiotic. Further, the ease with which many organisms may produce mutants resistant to an antibiotic reduces the usefulness of such a system. Incidentally, this system has been greatly abused for commercial purposes by the designation of various antibiotics as members of the "wide spectrum," "broad spectrum," "narrow spectrum," and "stubborn spectrum" groups.

PRACTICAL UTILIZATION

The practical utilization of antibiotics also has much to disqualify it as a basis for classification, since relatively few antibiotics produced by actinomycetes, among the many so far discovered, have found practical application. The favor which a particular antibiotic finds in the hands of the clinician or the veterinarian has little to do with its chemical nature and its biological origin.

CHEMICAL SYSTEM

The chemical system appears to offer the most logical basis for the classification of antibiotics of actinomycetes. Unfortunately, this system does not take into consideration the range of antimicrobial activities of the antibiotics; it does not explain the sensitivity and resistance of different microorganisms to what appear to be chemically closely related antibiotics; and it does not indicate

the practical potentialities of freshly isolated antibiotics.

In view of the rapidly accumulating information concerning the large number of actinomycetes capable of producing antibiotics under certain conditions, and in view of the numerous new antibiotics constantly being isolated in various laboratories throughout the world, no final system of classification can be proposed at the present time. The chemical nature of the substances, supplemented by a knowledge of the range of their antimicrobial activities, offers a good basis for a tentative system of classification, as outlined in detail in Part B.

Control and Standardization of Antibiotics

Those antibiotics that have found practical application in disease control, in animal feeding, or in the preservation of food materials are carefully controlled by official government agencies in different countries (Dony and Guisset, 1960). Official standards are usually established for such antibiotics, their sale for medicinal purposes being limited primarily to prescriptions. The wide and often indiscriminate use of antibiotics, by many physicians and especially in certain countries, has resulted in the rapid development of resistance to antibiotics in general and to some antibiotics, such as penicillin, in particular. This has led to recommendations that a careful study be made of the sensitivity of the causative agents of infection to various antibiotics, so that only a specific antibiotic would always be employed. Finally, the World Health Organization, recognizing the growing importance of antibiotics in the world at large, has taken steps to coordinate research in certain important problems pertaining to the wide use of antibiotics. Its Expert Committee on Antibiotics has recently (1961) published a report on standardization methods for assaying antibiotics.

Chapter 6

Chemical Nature of the Antibiotics of Actinomycetes

The known antibiotics produced by actinomycetes range in complexity from very simple compounds, such as nitropropionic acid (bovinocidin), to very complex proteins, such as the lytic enzymes of actinomycetin. Certain chemical types are linked with a specific range of antimicrobial activity. For example, the polyenes are mainly antifungal in nature, and the exceptions reported in the literature may very well be due to antibacterial impurities. Also the streptothricins are active against both gram-positive and gram-negative bacteria and even fungi, whereas the nonpolyenic macrolides are, as a whole, active only against gram-positive bacteria. In spite of these general correlations between types of structure and activity, it is not yet possible to predict in all cases the range of antimicrobial activity of an antibiotic purely on the basis of its chemical structure. Likewise, it is not yet possible to synthesize compounds, the biological properties of which would be known beforehand.

In this chapter, the antibiotics are grouped according to their chemical similarities. More detailed information and references will be found in Part B of this book and in the reviews written on this subject recently by Abraham and Newton (1958), Chain (1958), Van Tamelen (1958), Harman (1959), and also in the Merck Index (1960). From a chemical point of view, antibiotics can be grouped according to various criteria: (1) the elements contained in their molecules, (2) the most important groupings in these molecules, and (3) the structure of their molecular skeleton.

An examination of the empirical formulas of the antibiotics of actinomycetes listed in Part B of this book reveals that most of these substances contain carbon, hydrogen, oxygen, and nitrogen (153 compounds). Next in frequency are substances containing only carbon, hydrogen, and oxygen (31 compounds), followed by substances containing carbon, hydrogen, oxygen, nitrogen, and sulfur (18 compounds). This survey takes into account only those substances for which an empirical formula was at least proposed; however, their number is probably high enough to be representative of the whole group. A few substances have unusual elementary compositions: chloramphenicol, chlortetracycline, demethylchlortetracycline, and exfoliatin contain nonionic chlorine. Bromine can be substituted for this chlorine in chlortetracycline to form bromtetracycline, and possibly a similar substitution can be made in the other substances. Exfoliatin contains only carbon, hydrogen, oxygen, and chlorine, whereas the other antibiotics of this group contain, in addition, nitrogen. Two antibiotics have unique elementary composition: grisein contains sulfur and iron, and phleomycin contains

copper in addition to the four most common elements. One will note that there are no known antibiotics of actinomycetes with a molecule composed of only carbon and hydrogen.

In the following review of the chemical structure of the antibiotics of actinomycetes, an effort has been made to group together the antibiotics with similar structure. In some cases the antibiotics are grouped together because they have an outstanding functional group in common. For example, chloramphenicol is discussed with bovinocidin because they both have a nitro group.

In the chemical formulas used throughout this book no pretention is made to show the spacial arrangement of the atoms.

Nitro Compounds

Bovinocidin (β-nitropropionic acid) has the following structure:

This substance, which is also produced by higher plants and fungi, has weak antimycobacterial activity.

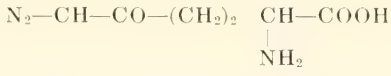
Somewhat more complicated chemically is *chloramphenicol*,

Its molecule, which contains nitrobenzene and two atoms of nonionic chlorine, is very active biologically. Chloramphenicol is active against both gram-positive and gram-negative bacteria, rickettsiae, and members of the lymphogranuloma group of intracellular parasites. It can be synthesized chemically in commercial quantities.

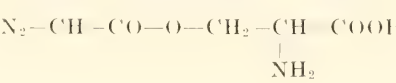
Azomycin, which is discussed under miscellaneous monocyclic compounds, is also a nitro compound.

Straight-chained Diazo Compounds

Azaserine and DON are diazo derivatives of amino acids. DON is 6-diazo-5-oxo-L-norleucine,

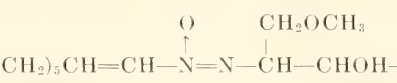


and azaserine is o-diazoacetyl-L-serine,



The antitumor activities of these two similar compounds have aroused interest. Both compounds also have modest antimicrobial activity. Three compounds of undetermined structure, *diazomycins A*, *B*, and *C*, are also aliphatic diazo compounds with a similar type of biological activity. *Alazopeptin*, a more complex substance, is a peptide containing α-alanine and a diazoketoamino acid. It has modest antitumor activity.

Elaiomycin is one of the few natural products with an aliphatic azoxy linkage:



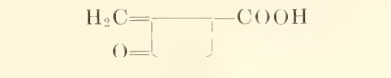
It is active almost exclusively against mycobacteria.

Miscellaneous Monocyclic Compounds

Questiomycin B is o-aminophenol,

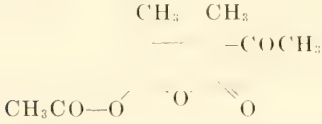
It has some antimycobacterial activity and is believed to be the building stone of another antibiotic, questiomycin A (6-aminophenoxazone), which will be discussed with the actinomycins.

Sarkomycin is a simple cyclopentane derivative,



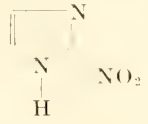
which has activity against tumor cells and a few gram-positive bacteria.

Acetomycin is a neutral saturated acetoxy lactone,



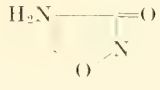
which is moderately active against mycobacteria and protozoa.

Azomycin is 2-nitro-imidazole,



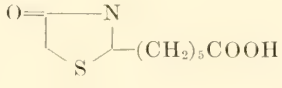
This antibiotic, which contains an equal number of molecules of nitrogen and carbon, is active mainly against bacteria.

Cycloserine is a ketoaminoisoxazole, which is active mainly against mycobacteria:



The chemical structure of cycloserine was identified as D-4-amino-3-isoxazolidone. On acid hydrolysis, it gives hydroxylamine and serine. Its biogenetic relationship to alanine has also been suggested (Abraham and Newton, 1958). Its synthesis has been brought about, as summarized by Van Tamelen (1958).

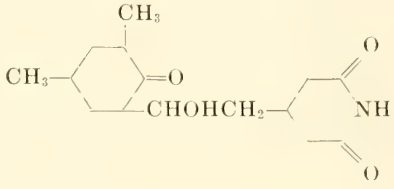
Another antibiotic with activity only against mycobacteria is *actithiazic acid*, a keto thiazole with an aliphatic acid chain in position 2:



It is 4-thiazolidone-2-caproic acid. It is soluble in organic solvents and its sodium salt is soluble in water. It has been synthesized, the racemate having about half the activity of the natural levo form. The toxicity is low, but the antibiotic is not active *in vivo* because of the presence of biotin, which interferes with its activity (Grundy *et al.*, 1952).

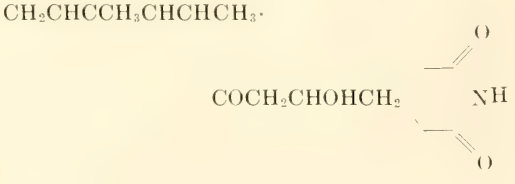
Cycloheximides

This is a group of antibiotics containing a glutarimide moiety. Of these, the antifungal agent *cycloheximide* has been known for the longest time:



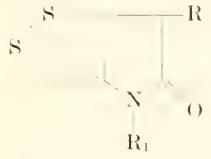
Its molecule has four centers of asymmetry. Some of the isomers of cycloheximide have been studied; they have been found to have less biological activity than the mother compound. One of the eight possible dehydrocycloheximides, *inactone*, has no biological activity. Four substances which have been studied mainly because of their antitumor activity, *streptovitacins A, B, and C* and *antitumor substance E 73*, have the same molecular skeleton as cycloheximide. Their structural formulas will be found in Part B of this book.

Streptimidone is an antifungal-antiprotozoal antibiotic which also has a glutarimide moiety to which an aliphatic chain is attached:



Aureothricin-type Compounds

Three antibacterial and antifungal antibiotics have the same molecular skeleton, composed of two rings formed of five atoms of carbon, two of sulfur, and one of nitrogen. The nitrogen-containing ring is of the pyrrolidine type:



Aureothricin: R = $NHCOCH_2CH_3$
$R_1 = CH_3$

Thiolutin: R = $NHCOCH_3$
$R_1 = CH_3$

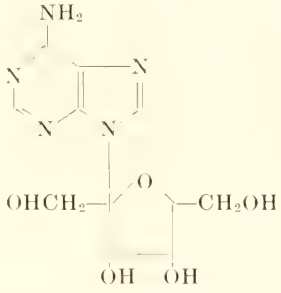
Holomycin: R = $NHCOCH_3$
$R_1 = H$

Aureothricin and *thiolutin* differ only in one CH_2 group in the side chain R. *Holomycin* is des-N-methyl thiolutin. Acid hydrolysis of aureothricin and thiolutin yields a weak amine, pyrrothine ($C_6H_6N_2OS_2$). The hydrolysis of holomycin yields des-N-methyl-pyrrothine, which is called holothin and which has some antibiotic activity of its own.

Other sulfur-containing compounds of different types were isolated from cultures of actinomycetes. They include lavendulin ($C_{49}H_{63}O_{18}N_{13}S_3$), sulfactin ($C_{27}H_{40}N_8O_5S_3$), thiostrepton (7.4 per cent S), and sulfocidin. Thioaurin ($C_{14}H_{12}N_4O_4S_4$) is an orange-yellow substance active against gram-positive and gram-negative bacteria but not against fungi.

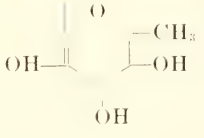
Compounds Containing a Purine or a Pyrimidine Base

A few products of actinomycetes contain a purine nucleus. They include puromycin, nucleocidin, psicofuranine, and the angustmycins. Three of these substances, *angustmycin C*, *antibiotic U 9586*, and *psicofuranine*, are presumed to have the same structure:



Their molecule contains two moieties, adenine (6-aminopurine) and the ketohexose, D-psicose. The special configuration of the atoms in the molecule of these three compounds must vary from one to the other, since their biological activity differs. Angustmycin C has no known biological activity. Antibiotic U 9586 has some activity against bacteria and tumors, and psicofuranine has some activity against tumors and bacteria *in vivo*. *In vitro* antibacterial activity was demonstrated when a special medium containing liver extract was used.

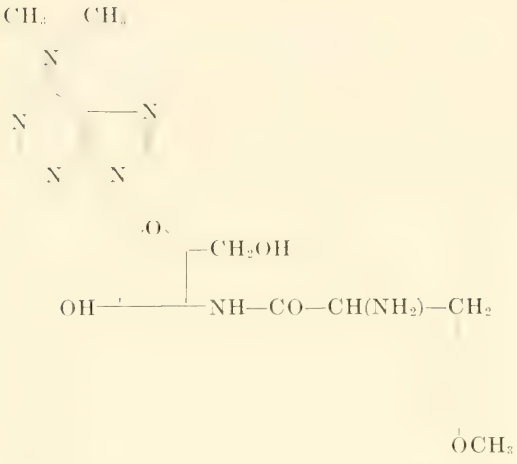
Angustmycin A differs from the previous compounds in the sugar attached to the 6-aminopurine, which is called angustose:



Angustmycin A is active mainly against mycobacteria.

Tubercidin and *toyocamycin* are similar compounds with an adenine and a D-ribose moiety.

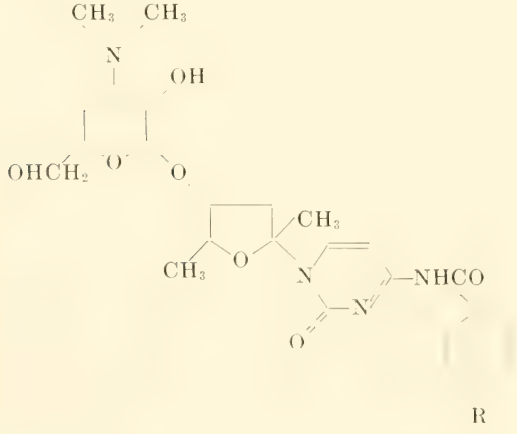
Puromycin has a more complicated molecule. It is composed of three moieties: (1) 6-dimethylamino purine, (2) D-3-amino-ribose, and (3) o-methyl-L-tyrosine:



Puromycin is active against bacteria (mainly gram-positive), protozoa, and tumors. Removal of the methyltyrosine moiety from the molecule caused a loss in antibacterial activity. The resulting aminonucleoside was, however, active against protozoa and tumors.

Nucleocidin is another member of this group of purine-containing compounds. It is active against bacteria and protozoa. It contains a 6-aminopurine moiety, a carbohydrate moiety, and sulfur.

Three antibiotics, *amicetin*, *amicetin B*, and *bamicetin*, are formed of four cyclic moieties, one of which is a pyrimidine: (1) *p*-aminobenzoic acid, (2) cytosine, (3) a six-carbon sugar-like unit in the furan form, and (4) a dimethylamino sugar, amosamine ($C_8H_{17}O_4N$):

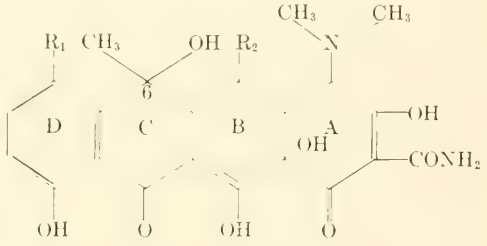


In the molecules of both amicetin and bamicetin,

The two antibiotics differ in that bamicetin has one less $—CH_2$ group in the glycosidic moiety than does amicetin. In amicetin B, R = NH_2. These three antibiotics are active mainly against gram-positive bacteria and mycobacteria. It is interesting to note that bamicetin is more active against gram-negative bacteria than are the amicetins. It has been suggested that amicetin B might be a precursor of amicetin.

Tetracyclines

The tetracyclines have in common a naphthacene nucleus; their range of biological activity approximates that of chloramphenicol; they are active against gram-positive and gram-negative bacteria, rickettsiae, and the psittacosis-lymphogranuloma group of organisms. The three commonly used antibiotics of this group are *tetracycline*, *chlortetracycline*, and *oxytetracycline*. They have the following formulas:



Tetracycline: $R_1 = R_2 = H$

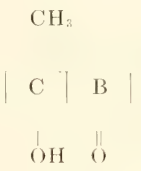
Oxytetracycline: $R_1 = H$; $R_2 = OH$

Chlortetracycline: $R_1 = Cl$; $R_2 = H$

When chlortetracycline-producing organisms are grown in chlorine deficient media that contain bromine, bromine is substituted for chlorine and bromtetracycline is formed. A slight drop in biological activity follows this substitution.

Mutants of *S. aureofaciens*, in chlorine-containing media, can form another useful variation of the chlortetracycline molecule by producing *demethylchlortetracycline*, in which there is no methyl group in position 6.

Anhydrochlortetracycline is obtained by acid degradation of chlortetracycline. It differs from chlortetracycline in the structure of rings B and C:



This compound has a rather specific activity against actinomycetes (Goodman *et al.*, 1955).

Antibiotic X 340 is a tetracycline, the complete structure of which is not yet known. It differs in biological activity from the other tetracyclines in that it is active mainly against gram-positive bacteria.

Acetylenic Compounds

Two antibiotics, cellocidin and mycomycin, have acetylenic bonds. *Cellocidin* is acetylenedicarboxamide,

$$H_2N{-}CO{-}C{\equiv}C{-}CO{-}NH_2$$

It is active mainly against mycobacteria, but also has a moderate action against bacteria.

Mycomycin has a wider range of antimicrobial activity, being active against bacteria, mycobacteria, and fungi. It is an unsaturated carboxylic acid with both ethylenic and acetylenic linkages:

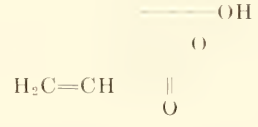
$$H{\equiv}C{-}C{\equiv}CCH{=}C{=}CHCH{=}$$
$$CH{-}CH{=}CHCH_2COOH$$

This highly unstable substance explodes at 75°C. A more stable and also biologically active isomer is obtained by treatment of mycomycin with alkali. It is called *isomycomycin*:

$$CH_2C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}$$
$$CH{-}CH{=}CH{-}CH_2{-}COOH$$

Polyenic Compounds

These unsaturated compounds have only ethylenic bonds. They form a large group of antibiotics which are mainly antifungal. These polyenes can be grouped together on the basis of the number of conjugated carbon to carbon double bonds present in the molecule. Most of them have four, five, six, and seven such unsaturated bonds in their chromophores which are responsible for very typical three-peaked light-absorption spectra. One antibacterial antibiotic, antibiotic PA 147, has a diene chromophore and could be considered as a small polyene:



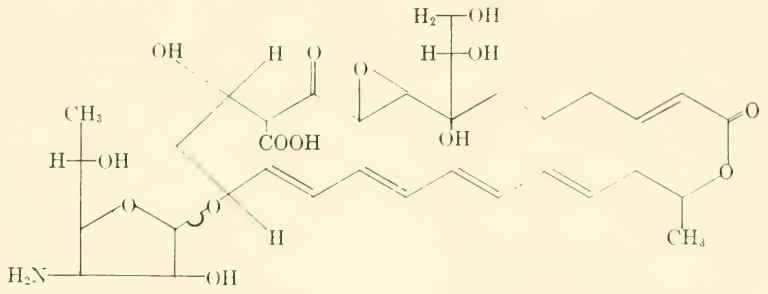
Antibiotic PA 147 has a lactone ring, like the larger polyenes, but the polyenic portion of the molecule is not all enclosed in the lactone ring.

The diene, antibiotic PA 147, has one single peak of light absorption at 272 mμ. The polyenes with four conjugated double bonds, the tetraenes, have three main peaks of light absorption; the position of the central peak is at 300 to 305 mμ. The position of the central peak is 330 to 340 mμ for the pentaenes, 355 to 359 mμ for the hexaenes, and 377 to 388 mμ for the heptaenes. To date, no triene or octaene has been discovered among the products of actinomycetes.

The complete structures of one tetraene, pimaricin, and two pentaenes, filipin and lagosin (Dhar *et al.*, 1960), have been reported. Some headway has also been made in the elucidation of the structures of the tetraene nystatin and of the heptaenes

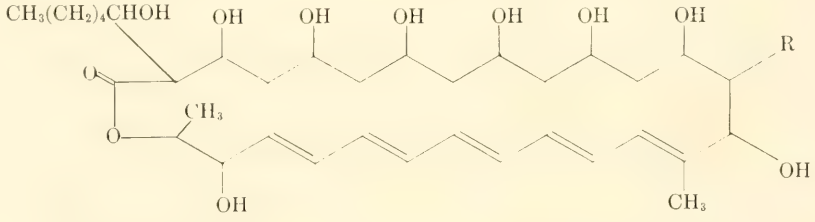
candidin, amphotericin B, candicidin, trichomycin, and perimycin.

Pimaricin has the empirical formula $C_{34}H_{49}NO_{14}$ and the following structure:



The nitrogen is present in the form of an amino group in a five-carbon sugar moiety, mycosamine. A large lactone ring of the type found in the bacterial macrolides, such as erythromycin, is formed in part by the tetraenic chromophore. A carboxyl and an epoxide group are attached to the large lactone ring. Mycosamine is also present in the molecule of another tetraene, nystatin, and in the molecule of some of the heptaenes. *Nystatin* has a larger molecule than pimaricin; it has the empirical formula $C_{46}H_{77}NO_{19}$.

Lagosin and *filipin* are two pentaenes which do not contain nitrogen:



Lagosin: R = OH

Filipin: R = H

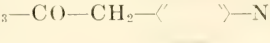
The large lactone ring of each is formed of 27 carbons and includes the pentaenic chromophore.

Other pentaenes, such as antibiotic PA 153, contain nitrogen.

The hexaenes are the least known polyenes. They have not been isolated as often as the tetraenes, the pentaenes, and the heptaenes. Of the five hexaenes reported in the literature, four are said to have some kind of antibacterial activity.

The chemistry of the heptaenes has been studied chiefly by Borowski and coworkers (1960). *Amphotericin B* and *candidin* are very closely related substances, each of which contains one atom of nitrogen located in the amino sugar, mycosamine. In contrast, candicidin, trichomycin, and perimycin have two amino groups per molecule. In candicidin and trichomycin, one of these amino groups is located in a mycosamine moiety and the other in a *p*-aminoacetophenone moiety:

Perimycin differs in that one of the amino groups is located in an amino sugar moiety of unknown structure and the other is in a p-aminophenylacetone moiety:



Perimycin, unlike other heptaenes, does not have a carboxyl group.

With the help of the keys, summaries of

the data published on the various polyenes can be found in Part B of this book. One should keep in mind that many of the polyenes described may be synonyms. This will be clarified by further studies in the chemistry of these interesting compounds.

Macrolides

Certain antibiotics, which are active mainly against gram-positive bacteria, are called macrolides because each has a large macrocyclic lactone ring. One will note that the polyenic macrocyclic lactones also have this structural feature. Since the nonpolyenic macrocyclic lactones were described first, the name "macrolide" must be reserved for this group of substances. However, the polyenic macrocyclic lactones can be called "polyenic macrolides."

TABLE 17

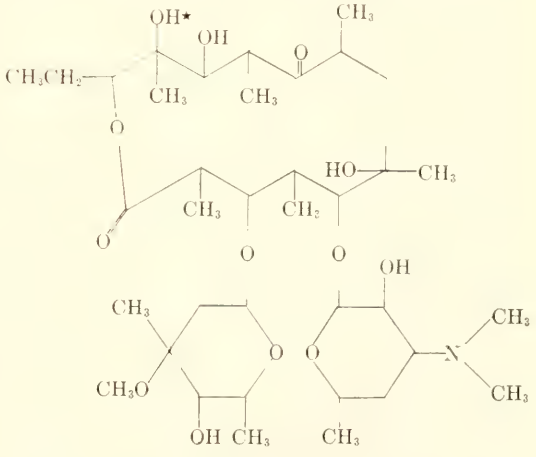
List of macrolide antibiotics

Antibiotic	Empirical formula
Amaromycin	$C_{25}H_{39}O_7N$
Angolamycin	$C_{49-51}H_{87-91}O_{18}N$
Antibiotic PA 108	$C_{38}H_{63}O_{14}N$
Antibiotic PA 133A	$C_{25}H_{43}O_6N$
Antibiotic PA 133B	$C_{25}H_{43}O_{10}N$
Antibiotic PA 148	$C_{38}H_{65}O_{15}N$
Carbomycin	$C_{42}H_{67}O_{16}N$
Carbomycin B	$C_{41-42}H_{67-69}O_{15-16}N$
Erythromycin	$C_{37}H_{67}O_{13}N$
Erythromycin B	$C_{37}H_{67}O_{12}N$
Erythromycin C	$C_{36}H_{65}O_{13}N$
Foromacidin D	
Griseomycin	$C_{28}H_{45}O_8N$
Leucomycin	$C_{33-38}H_{54-66}O_{11-13}N$
Leucomycin B	$C_{41}H_{69}O_{16}N$
Methymycin	$C_{28}H_{43}O_7N$
Miamycin	
Narbomycin	$C_{28}H_{47}O_7N$
Neomethymycin	$C_{28}H_{43}O_7N$
Oleandomycin	$C_{35}H_{61-63}O_{12}N$
Picromycin	$C_{28}H_{43}O_7N$
Proactinomycin A	$C_{27}H_{47}O_8N$
Proactinomycin B	$C_{28}H_{49}O_8N$
Proactinomycin C	$C_{24}H_{41}O_6N$
Spiramycin I	$C_{45}H_{78}O_{15}N_2$
Spiramycin II	$C_{47}H_{80}O_{16}N_2$
Spiramycin III	$C_{48}H_{82}O_{16}N_2$
Tylosin	$C_{45-46}H_{75-79}O_{17-18}N$
Tertiomycin A	$C_{42}H_{69}O_{16}N$
Tertiomycin B	$C_{41}H_{71}O_{17}N$
Teruchiomycin	$C_{28}H_{43}O_{16}N$

Picromycin was the first compound definitely known to belong to the macrolides, but the first antibiotics of this group to be isolated were the proactinomycins reported by Gardner and Chain in 1942 (see also Marston, 1949).

The macrolides are soluble in most organic solvents and, because of their amino sugar moiety, they are basic. Because of unsaturation in the molecule, most of these compounds absorb ultraviolet light. Table 17 indicates the variation in the size of the molecule from a carbon skeleton composed of 24 carbons atoms (proactinomycin C) to one composed of some 50 carbon atoms (angolamycin). The spiramycins are unique in that they contain two atoms of nitrogen per molecule.

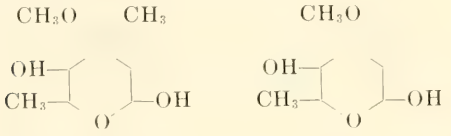
Erythromycin is the best known of the macrolides. It has the following structure:



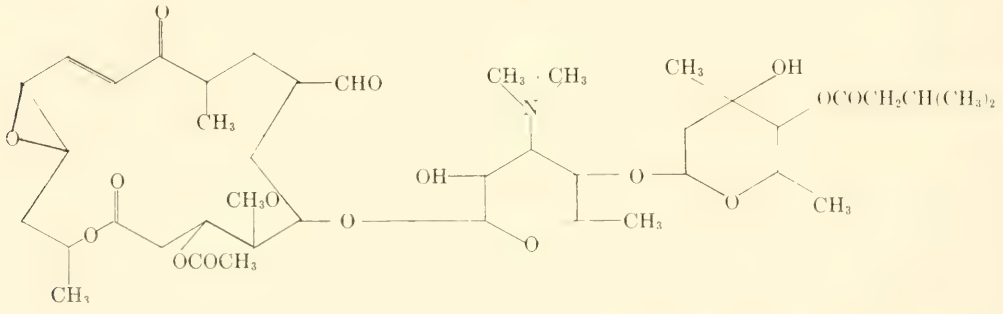
It contains two sugar moieties—a neutral sugar, cladinose, and an amino sugar, desosamine. *Erythromycin B* has a similar structure except that one of the hydroxyl groups (marked above with a star) is replaced by a hydrogen atom, and *erythromycin C* differs in that the cladinose moiety

is replaced by a $C_7H_{13}O_3$ fragment of unknown structure.

Oleandomycin is similar to erythromycin in that it contains desosamine, but its neutral sugar is oleandrose, which is closely related to cladinose. The structure of this antibiotic has recently been elucidated by Hochstein (1960).



Carbomycin contains a large lactone ring which is not fully saturated and which features an epoxide function adjacent to the unsaturated carbonyl function. Attached to this ring is the amino sugar mycaminose, to which is attached the neutral sugar mycarose:



Methymycin and *picromycin* do not contain a neutral sugar. They both have the same lactone ring, to which desosamine is attached. They differ only in the point of attachment of desosamine to the lactone ring. Methymycin has the following structure, the star indicating the point of attachment of desosamine in the picromycin molecule:

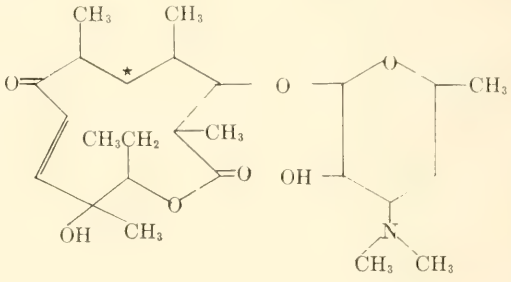


Table 18 lists some properties of tetraphenylboron derivatives of some macrolides. These derivatives can be used in the purification and characterization of these antibiotics.

Neomethymycin differs from methymycin only in the position of a hydroxyl group.

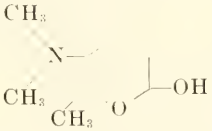
The exact structure of the *spiramycins*

TABLE 18

Tetraphenylboron derivatives of antibiotics (Zief *et al.*, 1957)

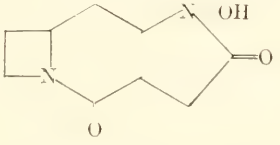
Antibiotic	Molecular weight	Nitrogen	Sodium tetraphenylboron derivative		
			m.p.	Nitrogen	
				Theory	Found
		%	°C	%	
Erythromycin	733	1.91	183–186	1.33	1.40
Carbomycin	830	1.69	148–153	1.22	1.20
Leucomycin	640–744	1.89–2.17	138–144	1.32–1.46	1.32
Oleandomycin	689	2.03	157–161	1.4	1.79

has not been elucidated, but they are known to be formed of a large macrolide ring to which is attached mycaminose and mycarose, already found in the carbomycin molecule, and which in addition contains another amino sugar moiety of the following structure:



Longisporin is an antibacterial antibiotic, the complete structure of which is not known. Preliminary data indicate the existence of a large multilactone ring (three lactone groups) of 36 carbons.

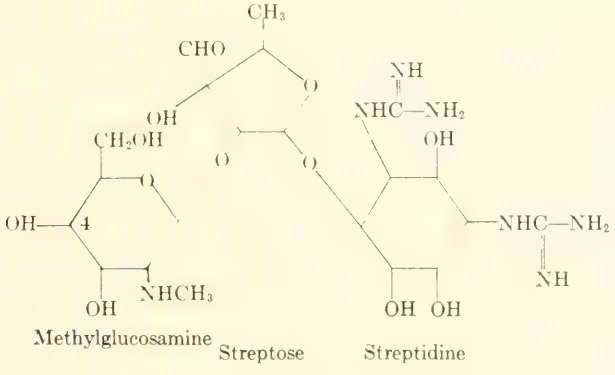
A compound which is somewhat similar to the antibacterial macrocyclic lactones is *nocardamine*. It has an odd molecular structure with a nine-membered ring with an adjacent three-membered ring. It has one weakly acidic and no basic center:



It is active only against mycobacteria.

Streptomycins

Streptomycin is active against gram-positive and gram-negative bacteria and mycobacteria. Its molecule is composed of three moieties—streptidine, streptose, and N-methyl-L-glucosamine:



Methylglucosamine Streptose Streptidine

Streptidine is a diguanido derivative of inositol. The hydrogenation of streptomycin under pressure, in presence of platinum oxide or palladium black, results in the reduction of the aldehyde group in the pentose, streptose. The resulting compound, which is biologically active and is chemically more stable than streptomycin, is called *dihydrostreptomycin* and can also be produced directly by certain *Streptomyces*. Another chemical transformation is the removal of an oxygen at the same site, with the formation of *dihydrodesoxystreptomycin*:

CHO—C—OH

Streptomycin

CH_2OH—C—OH

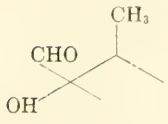
Dihydrostreptomycin

CH_2OH—CH

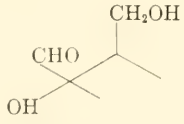
Dihydrodesoxystreptomycin

Streptomycin-producing strains of *S. griseus* form not only streptomycin but also a D-mannoside of streptomycin which was at first called streptomycin B and is now called *mannosidostreptomycin*. The mannose moiety is attached, in the pyranose form, to the 4 position of the N-methyl-L-glucosamine nucleus. Mannosidostreptomycin is only about one third as active as streptomycin.

Hydroxystreptomycin, which is produced by certain *Streptomyces*, differs from streptomycin in that it has a hydroxymethyl group instead of a methyl group in the streptose moiety:



Streptomycin



Hydroxystreptomycin

This antibiotic seems to offer no advantage over streptomycin.

Pseudostreptomycin is composed of two molecules of streptomycin linked together through their aldehyde groups by condensation with ammonia. The formula is thus:

$$SM\text{—}CHOH\text{—}NH\text{—}CHOH\text{—}SM$$

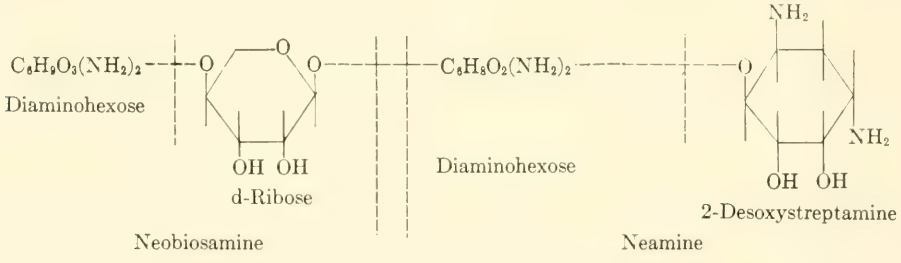
where *SM* stands for streptomycin minus the —CHO group. Pseudostreptomycin has little biological activity and is very toxic. It is converted to streptomycin in aqueous acidic solution.

Neomycins

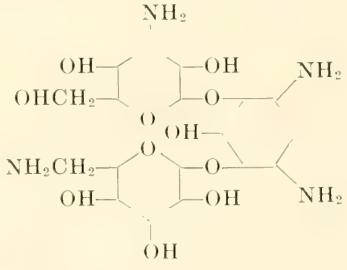
The neomycins are very similar to the streptomycins, but they do not contain guanido groups. They are formed of cyclic aminated moieties. These basic, stable, water-soluble substances are active against gram-positive and gram-negative bacteria and often have antiprotozoal activity.

Neomycin was first isolated in 1948 by Waksman and Lechevalier from a culture of *S. fradiae*. It was soon recognized that this antibiotic was made up of several chemical entities, the mixture being designated as the "neomycin complex." From this mixture, neomycins B and C were later isolated. An antibiotically active degradation product, neomycin A or neamine, produced in the culture or by chemical hydrolysis, was also obtained. The ninhydrin and other color tests, as well as specific test bacteria, can be utilized for the separation of the various neomycins.

Neomycin B is composed of four cyclic moieties, three of which have a carbon skeleton composed of six carbons and one of five carbons. One diaminohexose moiety is linked to the pentose, D-ribose, to form a C_{11} fragment called neobiosamine, and another molecule of a diaminohexose is linked to a diaminotrihydroxycyclohexane (2-desoxystreptamine) to form a C_{12} moiety called neamine. Neamine has some antibiotic activity of its own. Neobiosamine and neamine are linked together to form the C_{23} molecule of neomycin B, which has thus a total of six amino groups. *Neomycin C* is an isomer of neomycin B which is somewhat less active biologically. The difference between the two isomers is in the diamino hexose which is part of neobiosamine.



Kanamycin A is formed of three aminated cyclic moieties. These are 2-desoxystreptamine, which is also found in neomycin, and two aminohexoses:

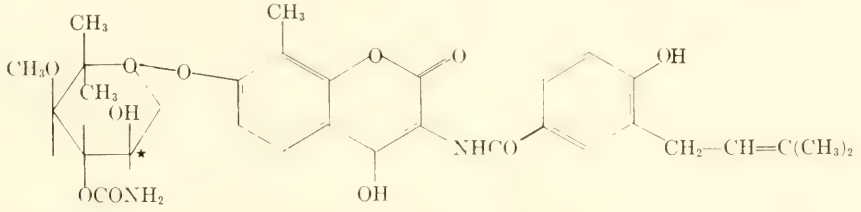


Other antibiotics of the "neomycin group" include *catenulin*, *paromomycin*, *hydroxymycin*, and *amminosidin*.

Miscellaneous Sugar-containing Compounds

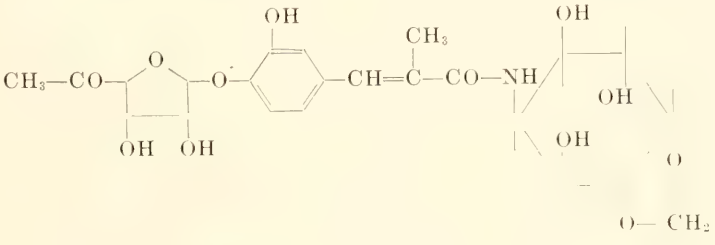
The simplest of the antibiotics of this group is *trehalosamine*. This antibiotic has the basic skeleton of the disaccharide trehalose, but in one of the glucose moieties a hydroxyl group is replaced by an amino group. Its biological activity is slight.

Novobiocin is an acidic antibiotic, the molecule of which consists of three moieties: (1) a sugar, (2) a substituted coumarin, and (3) a substituted benzoic acid:



The sugar, which is called noviose, includes a methoxyl and a carbamate group. In dilute alkali reaction, the carbamate group is shifted to the carbon indicated by a star to form an isomer of novobiocin, isonovobiocin, which is biologically inactive.

Hygromycin is a weakly acidic substance which is active against bacteria and is also toxic to worms. Its molecule is formed of three cyclic moieties: (1) an aminated inositol moiety which is believed to have a methylenedioxy group attached, (2) a hydroxylated and methylated cinnamic acid moiety, and (3) a hexose (5-keto-6-desoxyarabohexose):



Chartreusin is a weakly acidic, glucosidic, nonnitrogenous substance which is active against gram-positive bacteria and the exact structure of which is not yet known. Its molecule is formed of an aromatic nucleus containing three benzene rings linked to a disaccharide chain composed of D-fucose and D-digitalose.

Celesticetin is an amphoteric substance also active against gram-positive bacteria. Its acid hydrolysate yields a sulfur-containing base (desalicetin), salicylic acid, L-hygric acid, and a reducing amino sugar (celestose).

The *ristocetins* are mixtures of at least two compounds which are active mainly against gram-positive bacteria. Acid hydrolysis of the mixture yields D-arabinose, glucose, mannose, rhamnose, and a ninhydrin-positive substance.

Streptothricins

Streptothricin was the first basic, water-soluble antibiotic with activity against gram-positive and gram-negative bacteria to be isolated. Because of its toxicity it was never used clinically.

As explained in greater detail in Part B of this book, a large number of substances closely related to streptothricin have been isolated. All the "streptothricin type" antibiotics yield on hydrolysis a mixture of

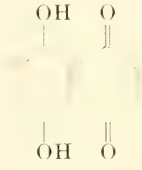
amino sugars and amino acids. Typical products of the hydrolysis of a streptothricin-type compound include (1) a diaminocaproic acid (L-β-lysine), (2) an imidazole derivative, streptolidine, and (3) the amino sugar α-D-gulosamine.

The streptothricins are active not only against bacteria but also against fungi. The most important streptothricin-type antibiotics are *lavendulin*, *actinorubin*, *antibiotic 136*, *roseothricin*, *geomycin*, *pleocidin*, and *mycothricin*. The antimycobacterial antibiotic *viomycin* also yields β-lysine upon hydrolysis.

Miscellaneous Pigmented Antibiotics which Act as pH Indicators

A number of antibiotics are colored substances which act as pH indicators, the color changing with the pH of their solution.

The structure of one of these substances, *actinorhodin*, is partially known. It has a dinaphthazarin nucleus. The structure of naphthazarin is:



Two such units are thus hooked together and are variously substituted.

Another pH indicator, *granaticin*, is a tricyclic tetrahydroxyquinonedicarboxylic acid ($C_{22}H_{20}O_{10}$).

Litmocidin, also a pH indicator, is believed to be related to the anthocyanin pigments. As such, it is believed to have a molecular nucleus of the following type:

The hydrolysates of another such indicator, *mycorhodin*, include reducing sugars. Nothing is known about the chemical structure of other pH indicators, such as *coelicolorin*, *mitomycin C*, *nocardorubin*, *rhodomycetin*, and *rubidin*.

The *rhodomycins* are a group of pH indicator antibiotics active against gram-positive bacteria. They are formed of a quinoid chromophore called rhodomycinon, to which is attached a moiety which contains rhodosamine, an isomer of desosamine.

Compounds Containing Phenazine or Quinoxaline Nuclei

One antifungal antibiotic, *1,6-dihydroxyphenazine*, and two antibacterial antibiotics, *griseolutein A and B*, have a phenazine nucleus which is substituted as follows:

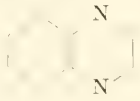
1,6-Dihydroxyphenazine: $R_1 = R_3 = H$; $R = R_2 = OH$

Griseolutein A: $R = OCH_3$; $R_1 = CH_2—O—CO—CH_2OH$; $R_2 = H$; $R_3 = COOH$

Griseolutein B: $R = OCH_3$; $R_1 = CH_2—O—CHOH—CH_2OH$; $R_2 = H$; $R_3 = COOH$

1,6-Dihydroxyphenazine is closely related to the bacterial antibiotic pigment iodinin, from which it can be obtained by reduction. Iodinin is the 5,10-dioxide of 1,6-dihydroxyphenazine and is active against bacteria. In contrast, 1,6-dihydroxyphenazine has no antibacterial activity. Both griseolutein A and B are active against gram-positive and gram-negative bacteria. The only difference between the two molecules is two hydrogen atoms in the R_1 chain. Despite this small difference, only slight cross-resistance was observed between these two antibiotics.

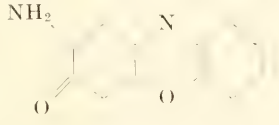
Echinomycin is an antibacterial antibiotic containing two quinoxaline moieties:



These are attached to a polypeptide moiety.

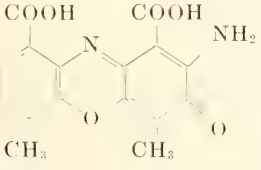
Phenoxazines: The Actinomycins

The *actinomycins* are red-colored antibiotics, the molecules of which consist of a phenoxazine nucleus to which are attached two polypeptide chains. These antibiotics are active against gram-positive bacteria and tumors. Actinomycins are usually produced by actinomycetes as mixtures of closely related compounds which all have the same phenoxazine chromophore but which differ in the amino acid composition of the polypeptide chains. *Questiomycin A* (6-aminophenoxazone) is active mainly against mycobacteria:



This rather nontoxic compound is closely related to the chromophore of the actinomycins. It is believed to be formed by the condensation of two molecules of questiomycin B (o-aminophenol).

The chromophore of the actinomycins is 3-amino-1,8-dimethylphenoxazone-(2)-dicarboxylic acid-(4,5):



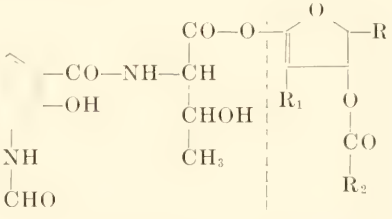
In the molecules of the actinomycins, two cyclic five-membered polypeptides are linked to the carboxyl groups. Their composition varies from one actinomycin to the other, as explained in Part B of this book.

The opening by alkali treatment of the polypeptide rings produces actinomycinic acids which have no antibacterial activity but some antitumor activities.

Some antibiotics other than the actinomycins are also polypeptides with an aromatic chromophore. They include *levomycin*, *mikamycin A*, *actinoleukin*, and as previously mentioned, *echinomycin*.

Oligopeptides

A group of closely related substances, the *antimycins* and *blastmycin*, have antifungal activity and inhibit electron transport in cytochrome systems. Their molecule is composed of two large moieties, antimycic acid and a neutral fragment. Antimycic acid itself contains two moieties: (1) L-threonine, and (2) a substituted salicylic acid fragment. The neutral fragment is composed of a variously substituted furan ring. The following structure has been suggested for these compounds:



Antimycic acid Neutral fragment

Blastmycin: $R = CH_3$; $R_1 = (CH_2)_3CH_3$;
$R_2 = CH_2—CH(CH_3)_2$

Antimycins: $R = C_7H_{15}$; $R_1 = H$;
$R_2 = CHCH_2CH_3$ (with CH_3 branch)

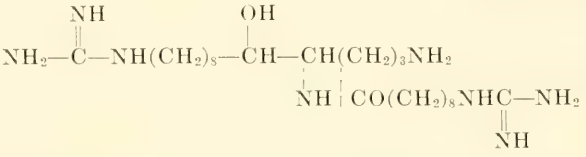
The various antimycins (there are at least four) probably differ in the structure of the C_7H_{15} fragment.

A recent paper (Strong *et al.*, 1960) has modified the concept of the structure of the antimycins. The substituted salicylic acid fragment, present in antimycic acid, is attached to a dilactonic ring.

Netropsin is active against both fungi and bacteria. Its nitrogen content is very high ($C_{18}H_{26}O_3N_{10}$), and it contains two pyrrole rings, a guanido group, two peptide groups, and a primary amino group as shown on the facing page.

A *prodigiosin-like substance* which is not so rich in nitrogen ($C_{25}H_{35}ON_3$) also contains two pyrrole rings.

Eulicin is a basic antifungal antibiotic which contains two guanido groups and a peptide linkage:

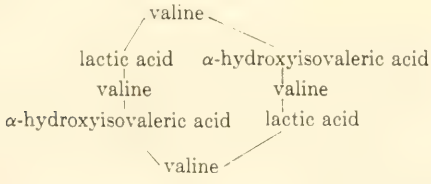


Acid hydrolysis of the base cleaves it, as indicated by the dotted line, and 9-guanidinononanoic acid and a base, eulicinine, are formed.

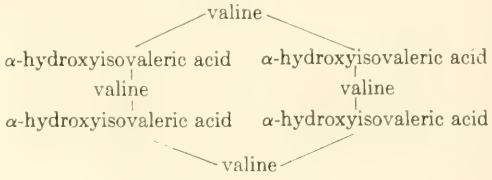
Polypeptides

We have already seen that some antibiotics have molecules which are partly polypeptidic in nature, such as the streptothricins and the actinomycins. A large number of antibiotics of actinomycetes are polypeptides *stricto sensu*. The exact structure of only a few of them is known.

Two rather simple polypeptides, amidomycin and valinomycin, have very similar molecules. *Valinomycin* is a cyclic polypeptide ($C_{36}H_{60}O_{12}N_4$) formed of the following units:

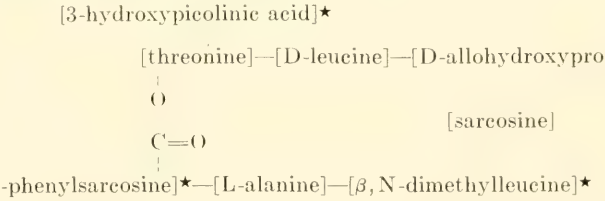


whereas *amidomycin* ($C_{40}H_{68}O_{12}N_4$) is formed of the following residues:



The only difference between the two antibiotics is thus four carbons and eight hydrogen atoms. Valinomycin was reported to have some activity against bacteria, and amidomycin to be antifungal in nature. Taber (personal communication) reported, however, that valinomycin also has antifungal activity.

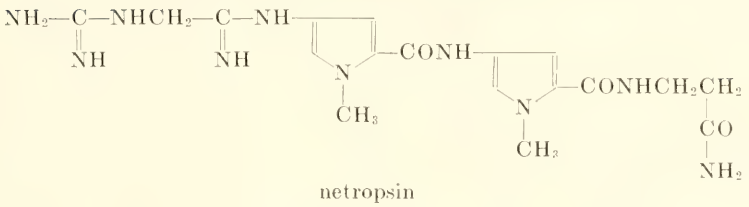
Etamycin is a macrocyclic peptide lactone which is active against gram-positive bacteria. Opening of the lactone ring results in the loss of antibiotic activity. In the following formula, the amino acids marked with a star had not been found previously in nature:



Staphylomycin S and *mikamycin B* are very similar to *etamycin*.

3-Hydroxypicolinic acid is also found in the molecule of *pyridomycin*, a polypeptide antibiotic which is active mainly against mycobacteria.

The complete structures of other polypeptide antibiotics of actinomycetes are not known. In the case of some of them the products of hydrolysis are known; of others, nothing is known except that they are polypeptides.



netropsin

Two of these substances stand apart because their molecules contain a metal. These are the iron-containing grisein and the copper-containing phleomycin.

Grisein and *albomycin* are mixtures of components. Hydrolysates of crude grisein and albomycin contain many amino acids. Purer preparations of grisein contain only glutamic acid and an unidentified amino acid, whereas a purified preparation of one of the albomycins contains only ornithine and serine. Removal of iron from the grisein molecule results in a loss of the yellow-orange color of the compound and in a reduction in its biological activity against gram-positive and gram-negative bacteria.

Phleomycin contains copper and is probably formed of two moieties, a polypeptide and a carbohydrate. This cobalt-blue compound, which is active against bacteria and mycobacteria, loses color upon removal of the metal, with a concomitant slight reduction in antimycobacterial activity.

Numerous other polypeptides are produced by actinomycetes, such as phalamycin, neocide, matamycin, amphomycin, cinnamycin, bryamycin, and thiostrepton.

In Part B of this book, a table lists the amino acids identified in the hydrolysates of antibiotics of actinomycetes and the antibiotic or antibiotics in which the acids have been found.

Proteins

The most chemically complex antibiotics are protein or protein-like substances, such as *actinomycetin*, various *bacteriolytic factors*, *cephalomycin*, and *micromonosporin*.

As this brief survey of what is known of the chemical structure of antibiotics of actinomycetes has shown, these filamentous bacteria have the ability to produce some intriguing compounds. The elucidation of the exact structure of an antibiotic, in complete stereochemical detail, is a type of work which heretofore has been mainly of academic interest. However, eventually a firmer link will be made between structure and mode of action, and chemotherapy will become a completely rational discipline.

Chapter 7

Biogenesis of Antibiotics

Only scattered attention has been paid heretofore to the mechanism of formation of antibiotics and to their role in the metabolism of the organisms producing these substances.

In this area, a number of questions can be asked which remain, for the most part, unanswered. Are antibiotics essential cell constituents? Are they storage products? Are they waste products of microbial metabolism? Are they a result of "abnormal" or "shunted" biosynthesis which may be controlled by certain special constituents of the medium? What role do they play in the life of the organisms producing them? What effect do they exert upon these organisms? Does the fact that they exert an inhibiting effect upon the growth of other microbes suggest that they play a role in the survival of the organisms producing them?

It has long been recognized that environment exerts a marked effect upon the composition of the microbiological population living in a natural or an artificial substrate. Important environmental factors include humidity, aeration, temperature, and pH as well as the nature and concentration of available nutrients. These factors also exert an important influence upon the metabolic processes and even upon the chemical composition, morphology, and life cycle of the organisms involved. In addition to factors of nutrition and environment, the nature of the microbial population also depends upon the presence of other organisms and upon their metabolic products. These exert a variety of associative or antagonistic effects upon the various members of the population. We have already discussed briefly the role of antibiotics in nature, and have concluded that it is doubtful that antibiotics play an important role in microbial ecology (Chapter 1). We will discuss here the part played by antibiotics directly on the cells of the producing organisms rather than indirectly, through their action on other microorganisms.

Role of Antibiotics in the Biology of the Producing Organisms

Let us begin this discussion by an examination of the effect of various factors upon the concentration of antibiotics produced by certain cultures of actinomycetes. Whether the active substance is excreted into the medium, whether it is liberated from the mycelium when the latter undergoes lysis, or whether it is extracted from the mycelium by means of special organic solvents during the isolation of the antibiotic are other important questions that must be considered.

In the production of actinomycin, for example, every change in the composition of the medium and in the cultural conditions brings about not only a change in the yield of the antibiotic, but also in the nature and ratios of its amino acid make-up. The synthesis of the mycelium and the yield of actinomycin show the same course. Both reach a maximum at a time of complete consumption of the carbon source; when

mycelial growth ceases, actinomycin no longer forms. Although the amount of actinomycin produced is small as compared to the mycelium synthesized, it is believed that the organism synthesizes, in addition to actinomycin itself, a large number of closely related compounds. It has been concluded that actinomycin is neither a degradation nor an autolytic product of the cell protein, but that its formation is a result of a side reaction of the assimilating process of metabolism (Martin and Pampus, 1956). Frommer (1957) found that phenylthiourea produces no depression in the respiration of organisms that produce actinomycin. The formation of this antibiotic in certain culture media is inhibited without growth inhibition.

Some of the antibiotics, such as streptomycin, neomycin, and the tetracyclines, are produced in concentrations of 5 to 10 gm per liter of culture medium. Although the media used for the production of these antibiotics vary greatly in chemical composition, one may assume that they contain about 30 to 80 gm per liter of nutrients, mostly carbohydrates, lipids, amino acids, proteins, and certain minerals. One may further assume that the organism metabolizes most of these nutrients, thereby converting about 30 per cent of the consumed substrate into cell substance. This would give a maximal synthesis of 10 to 25 gm of cell material per liter of medium. One can thus calculate that the antibiotic compounds make up approximately 33 to 40 per cent of the total cell material synthesized. In some cases, the transformation is undoubtedly much lower, as in the case of *Streptomyces antibioticus* growing in a glutamic acid-glycerol medium. Of the 15 gm of nutrients per liter, 10 gm are consumed in 10 to 12 days, giving 1.5 gm of cell material and 150 mg of actinomycin. The cell synthesis comprises only 10 per cent and antibiotic synthesis only 1 per cent of the nutrients added to the medium, or 1.5 per cent of the nutrients consumed.

Assuming the correctness of the above calculations, one is led to the inevitable conclusion that the antibiotic substance is not a mere metabolic intermediate or an ordinary waste product of the microbial cell, but that it is a constituent of the living cell protoplasm, a storage product, or a special by-product. If it were a storage product, the organism should have the capacity to utilize it for its own metabolism, which is not necessarily the case, although apparently mechanisms exist which bring about the destruction of at least some of the antibiotics if allowed to remain in contact with the living cells of the organisms producing them.

These calculations place such antibiotics as streptomycin beyond the scope of an intermediate or a waste product of metabolism. Thus the biogenesis of the antibiotic is closely allied to its role in the metabolism of the organism. Since so many different antibiotics are produced by different organisms, one must postulate a great variety of metabolic reactions. Further, since each organism frequently produces more than one antibiotic or several closely related chemical compounds, the question arises as to the extent and variety of metabolic reactions involved in the growth of actinomycetes.

Bu'lock (1960) emphasized the fact that although a single species or a restricted range of related species of microbes is able to produce a great variety of chemical compounds, often of great structural complexity, the fundamental chemistry of these organisms is based upon a limited group of reactions and compounds and "is remarkably uniform throughout most living things." We have thus, on the one hand, a limited variety of basic metabolic pathways; on the other, a seemingly endless variety of secondary metabolites, of which the antibiotics represent merely an arbitrary selection. As a result of recent studies on the biosynthesis of antibiotics and other natural products, it is possible to demonstrate that certain pre-

cursors (like the acetate or propionate types) of many well known secondary metabolites are also intermediates of primary metabolism. According to this concept, antibiotics are to be looked upon as "waste" products of the general metabolic reactions of the organisms.

Location of Antibiotics in the Producing Organism

The cells of bacteria and actinomycetes may be considered as being made up of three groups of constituents: cell walls, cell protoplasm, and slimy excretion products adhering to the cells, such as capsules. The protoplasm comprises: (1) functioning elements, notably nuclear material, enzymes and coenzymes, and other constituents that take part in the growth and multiplication of the cell; (2) metabolic constituents, including sugars, amino acids, fatty acids, vitamins, which are required for cell synthesis; (3) waste products of metabolism; and (4) storage or reserve materials (Holdsworth, 1952; Cummins, 1956; Cummins and Harris, 1956; Ikawa and Snell, 1956; Salton, 1956).

The question under consideration is: where do the antibiotics accumulate? The cell wall is certainly a logical place for them. Still, they may be considered as storage materials and even as metabolites. It is true that the organisms producing actinomycin or streptomycin are no longer able to utilize these antibiotics as nutrients. This may be due, however, to a change in the chemical structure of the compound after it has been isolated from the medium, since in the culture itself the substances tend to be destroyed after they have accumulated (see also Wiebull, 1956; Work, 1957).

As already mentioned, some antibiotics such as streptomycin are mainly found extracellularly in the culture medium. Others such as perimycin are found only in the mycelium of the producing organism. Still others such as candicidin are found in both locations.

These facts raise a number of questions which have not yet been successfully answered.

In our early studies on the formation of streptothricin, it was observed that when a culture broth was allowed to stand for several days at the end of the fermentation period, it tended to increase gradually in antibiotic potency. This suggested that streptothricin may have been originally bound to some chemical constituent in the cells of the organism; gradually this union was freed from its association with another compound by some enzyme system in the culture.

Eiser and McFarlane (1948) found that the presence of NaCl increased the permeability of the hyphae of *Streptomyces griseus* and allowed the liberation of streptomycin at a greater rate than in the absence of the salt. Only lysis of the mycelium served a similar purpose, since after prolonged incubation (6 days), the streptomycin in the medium without the salt had risen to the level of the medium with the salt.

Perlman and Langlykke (1950) found that large amounts of streptomycin occur bound in the mycelium of the organism producing the antibiotic. They suggested that the antibiotic may be a part of the cell wall of the actinomycete. The bound streptomycin could be released by treatment of the cells with acid, alkali, or ionizable salts, as well as by sonic vibrations. Perlman (1953) reported that various other antibiotics, notably neomycin and chloramphenicol, were also bound to the mycelium of the organisms producing them and could be released by various treatments. The binding of the antibiotic was not a simple ion exchange phenomenon, since addition of streptomycin to the mycelium did not result in adsorption of the antibiotic. Although in our early studies NaCl was observed to have a favorable effect upon the "production" of streptomycin, this effect was explained as the result of the freeing of the

"bound" antibiotic. Actually, higher production of streptomycin may be obtained in the absence of salt.

Gwatkin (1954) found that large quantities of neomycin, sufficient to account for nearly all the antibiotic which later appeared in the medium, had been present in the mycelium of the organism producing this antibiotic (*S. fradiae*). At a proper pH, the neomycin is released by salt from its combination, which was considered to be a neomycin-nucleic acid complex. This complex was found to be present in the disintegrated cell material. Neomycin formed insoluble compounds with ribonucleic and deoxyribonucleic acids and protamine nucleinate, but not with nucleotides or hydrolyzed nucleic acid. Neamine, a hydrolytic product of neomycin, did not form any such complexes.

Surikova and Rudakova (1958) compared various methods of extraction of streptomycin from the mycelium of *S. griseus*. The most effective method consisted in the acidification of the mycelium with a mineral acid (pH 2.5) and subsequent heating. As much as 13 per cent of the streptomycin could thus be extracted, as compared to the amount obtained from the broth. In the case of a soya bean medium, it was possible to extract about 15 per cent of the streptomycin. The amount of the antibiotic bound with the mycelium of the organism, as calculated per unit weight of mycelium, did not change significantly in the process of fermentation.

Legator and Gottlieb (1953) found that chloramphenicol production reached its peak well after maximal growth had been attained. This was particularly true of organic media, a rise in the concentration of the antibiotic being correlated with an increase in pH and in ammonium ions, and finally with a lysis of the mycelium. These investigators believed that the antibiotic is not stored within the cells of the organism to any large extent. It is either immediately secreted into the medium upon production, or is formed from a degradation product. When high concentrations of the antibiotic were added to the medium, no more chloramphenicol was formed, the concentration needed to arrest further production being equivalent to the amount normally formed by the cell when no antibiotic was added. Chloramphenicol added at any time during the growth phase of the organism exerts its limiting action on further production, although not on the synthesizing ability of the cells.

These data indicate that most antibiotics are formed intracellularly and are released in the culture medium. It is doubtful that any of the well known antibiotics are formed extracellularly.

Mechanism of Biosynthesis of Antibiotics

On the basis of what is known of the chemical structure of antibiotics of actinomycetes and of the mechanism of their biosynthesis, Abraham and Newton (1960) grouped antibiotics into three main classes: those derivable from sugars, from amino acids, and from acetate. In the following discussion we have grouped the antibiotics according to this general outline.

Antibiotics Derivable from Sugars

STREPTOMYCIN

It has been definitely established that some of the nutrients in the medium are important largely for cell growth and others for antibiotic synthesis. This has been studied extensively for penicillin, streptomycin, and the tetracyclines. Agatov and Kazanskaya (1958) have shown that in the growth of streptomycin-producing *S. griseus*, during the first 2 days, histidine and arginine are rapidly utilized and lysine is utilized more slowly; on the third day, rapid utilization of lysine takes place. This is accompanied by

liberation of nucleic acids and monoamino acids into the medium. When alanine is present in the medium, basic amino acids and nucleic acid metabolites appear in the first 2 days; on the following day the concentration of histidine and nucleic acid metabolic products diminishes. Arginine and lysine remain on the same level. Inositol and substances containing a guanidine grouping exert a marked effect upon the biosynthesis of streptomycin (Egorov, 1959).

Shaposhnikov *et al.* (1959) made a study of the formation of streptomycin in synthetic media containing proline, or histidine with lysine or proline, succinimide, or succinamide. The yield of antibiotics on these media was 74 to 84 per cent of that on soybean meal medium. Oxyproline was found to be a unique source of nitrogen, contributing to the growth of the actinomycete, while only weakly stimulating the formation of streptomycin (Table 19). When this amino acid was added to a medium already containing the basic amino acids, growth was favored but the antibiotic yield was lowered (Table 20).

Schaiberger (1959) examined in detail the mechanism leading to the biosynthesis of streptomycin. He used a high streptomycin-producing culture of *S. griseus* (S+) and a mutant without streptomycin (S−) derived from it. The S− culture was asporogenous and lacked the ability to synthesize streptomycin; however, its rates of growth and of sugar utilization were double those of the S+ strain. The nutritional requirements for growth and streptomycin synthesis by the S+ culture included glucose, a suitable inorganic nitrogen source (ammonia), and six mineral salts ($MgSO_4$, $FeSO_4$, $ZnCl_2$, $CaCO_3$, K_2HPO_4, and NaCl). These nutrients also satisfied the growth requirements of the S− strain. Resting mycelial suspensions confirmed the essentiality of the salts for streptomycin synthesis, with the exception of K_2HPO_4. Exogenous addition of phosphate inhibited streptomycin synthesis by resting cells. When used as the sole nitrogen source in a mineral salts-glucose medium, proline and the amino acids closely related metabolically (asparagine, histidine,

TABLE 19

Maximal mycelial weights and quantities of streptomycin on media with pyrrole compounds (Shaposhnikov *et al.*, 1959)

Medium	Mycelial weight	Amount of streptomycin		
		μg/ml	% of control I	% of control II
	mg/100 ml			
Control I*		1200	100	1070
Control II	360	112	9	100
Pyrrolidine	446	524	44	468
Proline	738	1312	109	1171
Oxyproline	738	328	27	293
Succinimide	327	206	17	184
Succinamide	286	190	16	170

* Control I contains an inorganic nitrogen source. Control II contains an organic nitrogen source. Both controls have soybean meal added.

TABLE 20

Maximal mycelial weights and quantity of streptomycin on media with histidine, lysine, and pyrrole compounds (Shaposhnikov *et al.*, 1959)

Medium	Mycelial weight	Amount of streptomycin		
		μg/ml	% of control	Amount of streptomycin in control
	mg/100 ml			*μg/ml*
Basic medium*	646	999	53	1869
Pyrrolidine	689	916	68	1340
α-Methylpyrrolidine	769	1163	74	1568
Succinimide	592	1575	84	1870
Succinamide	725	1524	81	1874
Oxyproline	806	744	48	1550
Proline	797	1310	75	1743

* Basic medium: 2% glucose, 0.3% $(NH_4)_2SO_4$, 0.25% NaCl, 0.05% KH_2PO_4, 0.3% $CaCO_3$, distilled water. Soybean meal, amino acids, or other pyrrole compounds added on basis of 112 mg of nitrogen per 100 ml of medium.

and glutamic acid) permitted the highest level (1000 to 3000 μg per ml) of streptomycin synthesis by the S+ culture. Norleucine and isoleucine strongly inhibited streptomycin synthesis in experiments with growing cells as well as with resting cells; leucine stimulated synthesis. Resting cell data also suggested that acetate is an intermediate in streptomycin biosynthesis.

Silverman and Rieder (1960) utilized the distribution of radioactivity among the individual carbon atoms to elucidate the mechanism of the formation of N-methyl-L-glucosamine in the streptomycin molecule from D-glucose. Both D-glucose-1-C^{14} and D-glucose-6-C^{14} were employed. A method for the isolation of the methyl glucosamine was described, as was a procedure for the degradation of this amino sugar. The conclusion was reached that the major portion of the radioactivity incorporated into the carbon chain of the amino sugar was in carbon 1 when D-glucose-1-C^{14} was used and in carbon 6 when D-glucose-6-C^{14} was employed.

These investigators suggested that a possible mechanism for the inversion of all the asymmetric carbons of D-glucose is one of multiple epimerizations. Other possibilities were also suggested, such as an extensive series of oxidations and reductions with subsequent inversion of configuration upon reduction, or a mechanism of multiple isomerizations.

The changes occurring during streptomycin production are shown in Table 21. Little progress has so far been made in the use of precursors for streptomycin biosynthesis. By using C^{14} compounds, it was shown (Hunter *et al.*, 1954) that the guanidine carbon is derived largely, if not entirely, from CO_2, and that compounds such as arginine may act as precursors. The streptamine and streptose portions of the streptomycin molecule appear to be formed from glucose (Hunter and Hockenhull, 1955). Streptamine itself does not act as a precursor, but N-methyl-L-glucosamine does. According to Egorov (1957), a combination of a guanidine compound (L-arginine, creatine, guanidine) and inositol favors the biosynthesis of streptomycin. Asparagine favors growth but not streptomycin synthesis. Hydrolysates of casein and soybean

TABLE 21

Changes occurring during fermentation of glucose-meat extract-peptone medium
(Dulaney and Perlman, 1947)

Medium: Glucose 1 per cent, meat extract 0.5 per cent, peptone 0.5 per cent, sodium chloride 0.5 per cent.

	Duration of fermentation (days)								
	0	1	2	3	4	5	6	7	8
Mycelium (mg/ml)		0.4	5.1	5.8	5.7	4.8	4.6	4.2	3.8
Streptomycin (mg/liter)		0	37	194	198	231	270	186	267
Glucose (mg/ml)	9.0	8.8	8.0	2.4	1.2	0.6			
Soluble C (mg/ml)	10.2	8.6	7.0	5.1	5.0	4.4	4.6	4.5	4.6
Lactic acid (mg/liter)	292	328	114	13	10	16	12	6	15
Oxygen demand (Q_{O_2}/ml)		19	81	82	53	25	5		
Soluble N (mg/ml)	1.48	1.30	1.10	0.67	0.70	0.73	0.90	0.88	1.14
Mycelial N (mg/ml)		0.04	0.44	0.62	0.57	0.49	0.40	0.38	0.29
Inorganic P (mg/ml)	118	108	34	1	5	2	19	24	34
Ammonia N (mg/liter)	66	70	75	63	103	115	179	232	265
pH	7.35	7.30	7.55	7.50	7.75	8.25	8.55	8.65	8.90

TABLE 22

Changes characterizing the three phases of streptomycin production (Hockenhull, 1960)

	Phase 1: "growth"	Phase 2: "maturation"	Phase 3:"senescence"
Streptomycin	Slight production	Maximal rate of production	Streptomycin level ceases to rise or falls
pH	Steady rise	Very slow fall	Rise
Mycelium	Rapid growth	Mycelial weight fairly constant	Mycelial disintegration
Glucose	Used slowly	Used steadily throughout	Usually absent
Ammonia	Released into medium	Utilized	Released
Inorganic phosphate	Released	Utilized	Released
Q_{O_2}	High	Moderate	Low
Total oxygen demand	High	High	Low

meal are favorable for the production of the antibiotic.

A detailed review of the biogenesis of streptomycin has recently been published by Hockenhull (1960). Three distinct phases were recognized in the growth of the organism and formation of the antibiotic (Table 22). Although the surface growth of the streptomycin-producing *S. griseus* tended at first to give higher yields than the growing of the organisms under submerged conditions (Thornberry and Anderson, 1948), the latter method gradually became generally employed. The first medium, recommended by Waksman and Schatz (1945), consisted, in grams per liter, of glucose (10), peptone (5), meat extract (5), and sodium chloride (5). Yeast extract, soybean meal, and dried whole yeast were later used to replace the meat extract. Glucose was usually the sugar of choice, in amounts of 10 to more than 25 gm per liter. Certain strains were found to be able to utilize, for streptomycin production, fats, oils, or certain fatty acids in place of glucose (Perlman and Wagman, 1952).

Of the various nitrogen sources, L-proline was found (Table 23) to be the most effective for streptomycin synthesis, although it is only slowly utilized for the growth of the organism.

Streptomycin is basically a trisaccharide with various substituent nitrogen groups. By the use of isotopic carbon (C^{14}), Hunter and Hockenhull (1955) demonstrated that the carbon of the glucose was distributed evenly among the streptamine, streptose, and the N-methyl-L-glucosamine portions of the streptomycin molecule. The carbon of the guanidine group was poorer in radioactivity, thus indicating that this carbon came from CO_2, as shown in Table 24 (see also Numerof *et al.*, 1954).

The effect of phosphate concentration on streptomycin production is illustrated in Table 25. In synthetic media, phosphate exerted an effect on glucose breakdown and on streptomycin biosynthesis. An excess of phosphate caused an increased glucose consumption. Increasing concentrations of phosphate first showed an increase, then a decrease in streptomycin synthesis. Arsenate had a similar effect (Hockenhull *et al.*, 1954).

Hockenhull (1960) concluded that the following factors favored streptomycin biogenesis: (1) high oxygen supply, (2) low inorganic phosphate, (3) adequate glucose concentration, and (4) nitrogen levels that would not lead to high protein synthesis. It was suggested that once the enzymes required for streptomycin production have been formed, further biosynthesis will take

TABLE 23

Effect of organic nitrogen compounds on streptomycin production (Dulaney, 1948)

Compound added (0.1%)	Streptomycin produced: Glucose diammonium hydrogen phosphate medium	Streptomycin produced: No other nitrogen source
	μg/ml	*μg/ml*
DL-Alanine	155	234
Glycine	116	151
L-Arginine-HCl	135	101
L-Aspartic acid	125	12
L-Cysteine HCl	0	0
L-Cystine	105	0
Creatine hydrate	163	0
L-Glutamic acid	115	4
L-Histidine HCl	145	112
Hydroxy-L-proline	135	1–2
DL-Isoleucine	127	39
L-Leucine	137	5
DL-Lysine HCl	139	0
DL-Methionine	84	0
DL-Norleucine	0	0
DL-Phenylalanine	166	0
L-Proline	175	800
DL-Serine	155	3
DL-Threonine	110	5
DL-Tryptophan	129	0
L-Tyrosine	176	0
DL-Valine	168	3
Urea	136	3
Guanidine nitrate	133	5
0.1% Corn steep solids	300	
0.1% Casein digest	249	
Diammonium hydrogen phosphate		166
None	153	

place without the formation of new cells. Hockenhull (1960) concluded that streptomycin "does not constitute a portion of the wall polymers and certainly was not a major constituent."

Streptomycin is a glycoside containing the monosaccharides streptose and N-methylglucosamine, whereas mannosidostreptomycin contains streptose, N-methylglucosamine, and mannose. Both antibiotics are present in the broths of *S. griseus*. The enzymatic conversion of mannosidostreptomycin to streptomycin, which involves hydrolytic removal of the mannose unit, is an important step in the production of streptomycin.

Glucose preferentially enters the streptomycin molecule. A threefold increase in specific radioactivity has been observed in the streptomycin produced in a medium containing labelled glucose as compared with the specific radioactivity of the carbon in the nutrients supplied (Karow *et al.*, 1952). The supplementation of *S. griseus* broth with mannose has resulted in an increase in the proportion of mannosidostreptomycin. Mannose, therefore, meets a second criterion for

TABLE 24

Conversion of C^{14}-glucose to streptomycin (Hunter and Hockenhull, 1955)

Medium: soya bean meal-distillers' solubles-glucose. Glucose added 60 hours after inoculation.

Substance	Specific activity	
	μc/m-mole	*μc/mg carbon*
Glucose	9.1	12.6×10^{-2}
Streptomycin	29	11.6×10^{-2}
Streptidine	9.9	10.4×10^{-2}
Streptamine	8.8	12.3×10^{-2}
$BaCO_3$ (from guanidine groups)	0.55	4.5×10^{-2}
N-Methyl-L-glucosamine	10.1	12.2×10^{-2}
Streptose (by difference)	9.2	12.8×10^{-2}

TABLE 25

Streptomycin production in a proline medium, with amount of diammonium hydrogen phosphate as variable (Woodruff and Ruger, 1948)

$(NH_4)_2HPO_4$ concentration	Streptomycin (on 8th day)
mg/ml	*mg/liter*
5.7	225
2.0	170
0.60	570
0.20	675
0.06	530
0.02	390
0.006	200
0.002	130
Nil	25

controlled biosynthesis in that it modifies fermentation to result in the formation of an antibiotic which contains mannose as an integral unit. Mannose is known, however, to inhibit the action of the enzyme mannosidase, which converts mannosidostreptomycin to streptomycin (Hockenhull *et al.*, 1954). The formation of mannosidostreptomycin in a medium supplemented with mannose is the result of controlled biosynthesis; the organism has been induced to synthesize a biologically undesirable compound rather than an active antibiotic. The controlled biosynthesis technique may thus be employed strategically to design new molecules with desired chemical properties.

The biosynthesis and the degradation of mannosidostreptomycin, especially the formation and action of the enzyme α-mannosidase, were also studied by Abalo and Varela (1960).

Further information on biosynthesis of streptomycin is found in the work of Hunter (1956).

NEOMYCIN

Sebek (1955) observed that when glucose labelled uniformly with C^{14} was added to a growing culture, in a medium containing 9 gm of glucose per liter, 19.5 per cent of the carbon of the sugar was incorporated in the neomycin. The rest was distributed in the CO_2, in the filtrate, and in the mycelium. The fact that the antibiotic was also readily produced in the presence of other sugars, including mono-, di-, and polysaccharides, pentoses, hexoses, and sugar acids, suggested the operation of a general basic mechanism of sugar breakdown and antibiotic synthesis.

Antibiotics Derivable from Amino Acids

ACTINOMYCINS

An actinomycin-producing organism generally synthesizes a mixture of different actinomycins. *S. antibioticus*, for example, gives a mixture of actinomycins I to V; occasionally, trace amounts of a sixth component are also formed. *S. chrysomallus* produces actinomycins IV, VI, and VII. The quantitative and qualitative nature of the mixture synthesized can be modified to a considerable degree by modifying the medium in which the organism is growing. The nitrogen source in particular was found to influence the composition of a given mixture of actinomycins. Actinomycin IV increased from 10 to 83 per cent of the complex produced by *S. chrysomallus* when DL-valine was added to the medium; when DL-isoleucine or sarcosine was added, new actinomycins were formed (Schmidt-Kastner, 1956). Hydroxy-L-proline brought about an increase in the synthesis of actinomycin I from 6 to 7 to 31 per cent of the complex produced by *S. antibioticus* (Katz and Goss, 1958).

Katz (1960) made a detailed study of the effect of addition of sarcosine upon the synthesis of actinomycins II and III by *S. antibioticus*. The formation of these two actinomycins was found to depend, in part, on the concentration of sarcosine and on the time and the number of additions of this amino acid. The effect of sarcosine was highly specific, compounds structurally and biochemically related to it being ineffective. The actinomycins were found in both the mycelium and the medium. The addition of L-proline reversed the effect of a given concentration of sarcosine; larger amounts of sarcosine nullified the effect of proline. Incorporation of DL-pipecolic acid, a proline analog, into the medium resulted in synthesis of several new actinomycins. When washed suspensions of *S. antibioticus* were incubated in the presence of 1 m*M* sarcosine, there was a five fold increase in the synthesis of actinomycin III but no change in that of actinomycin II.

Schmidt-Kastner (1956) suggested that sarcosine interferes with the incorporation

TABLE 26

Neutralization by DL-threonine of DL-isoleucine inhibition of actinomycin production (Kawamata *et al.*, 1960)

Incubation	Actinomycin production		
	Control	DL-Isoleucine 100 μg ml	DL-Isoleucine 100 μg ml + DL threonine 100 μg/ml
days			
2	0	0	0
4	100	0	200
6	400	0	400
7	2000	0	800
8	4000	0	1000
9	4000	0	4000

of proline into certain actinomycin peptides. The results obtained support the view that sarcosine competes with and replaces proline in the peptide of certain actinomycins (Katz and Goss, 1958).

Kawamata *et al.* (1960) found that addition of DL-isoleucine to the medium (starch-glutamate-salts) represses the formation of actinomycin, whereas growth of the organism is not adversely affected and is even stimulated. The addition of 1 mg per ml of isoleucine brought about complete inhibition of antibiotic production. The addition of DL-threonine to the medium completely reversed this repressive effect, as shown in Table 26.

Antibiotics Derivable from Acetate

ERYTHROMYCIN

Erythromycin has two sugar-like groups attached to a large lactone nucleus. The lactone nucleus is made up of seven propionic acid units (Woodward, 1957). Acetic, propionic, and valeric acids may be incorporated into the lactone ring. This is true especially of propionic acid, a fact which suggests that this acid is an important precursor, and that the other two acids are converted into it before incorporation.

Gerzon *et al.* (1956) suggested that the macrolide ring arises, at least in the erythromycins, by a process analogous to that by which many long-chain molecules are built from acetate, but utilizing propionate instead. The long carbon chains of such antibiotics as the methymycins, picromycin, and narbomycin would be derived in essentially the same way except for incorporation of one acetate moiety.

Musílek and Ševčík (1958a) demonstrated that the addition of sodium arsenite (final concentration of 4×10^{-4} *M*) to the fermentation medium reduced the biosynthesis of erythromycin by *S. erythreus* by 87 per cent, whereas it increased the accumulation of pyruvic acid (Figs. 1 and 2). The addition

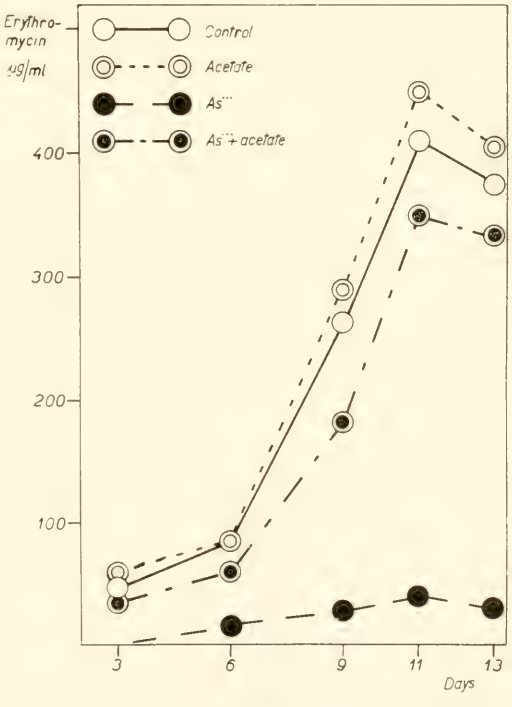


FIGURE 1. Inhibition by arsenite of erythromycin biosynthesis in submerged cultivation of *S. erythreus*. (Reproduced from Musílek, V. and Ševčík, V. Folia Biol. 4: 319–327, 1958b.)

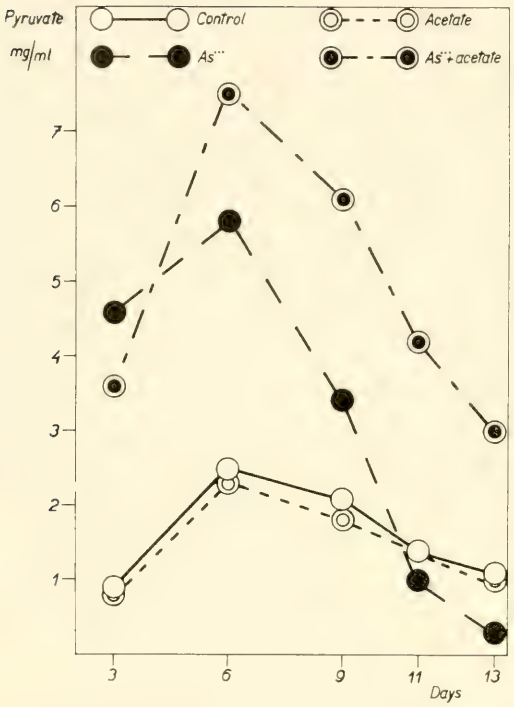


FIGURE 2. Effect of arsenite on the accumulation of pyruvate in a submerged culture of *S. erythreus*. (Reproduced from Musílek, V. and Ševčík, V. Symposium on antibiotics, Prague Abstr., p. 32, 1959.)

of 0.5 per cent sodium acetate or propionate markedly reduced the inhibitory effect of arsenite on the biosynthesis of erythromycin. Other salts of organic acids, as well as glycine, failed to nullify the effect of arsenite. The latter completely inhibited the oxidative decarboxylation of pyruvate and oxidation of acetate by washed suspensions of *S. erythreus*. Oxidation of glucose was only partially inhibited. It was therefore concluded that the biosynthesis of erythromycin is dependent on the oxidative decarboxylation of pyruvic acid to acetic acid. The participation of acetic acid as the initial substrate for the biosynthesis of propionic acid, presumed to be the precursor of the lactone nucleus of the erythromycin molecule, was suggested (Musílek and Ševčík, 1958b).

According to Musílek *et al.* (1958), the biosynthesis of acetylmethylcarbinol depends largely on the composition of the medium and on the presence of arsenite, which regulates the metabolism of pyruvic acid. In the case of *S. aureofaciens*, the stimulating effect of phosphate on the production of acetylmethylcarbinol suggested a partial explanation of the negative effect of phosphate on the biosynthesis of chlortetracycline (see also Biffi *et al.*, 1954). The addition of arsenite to a submerged culture of *S. erythreus* reduced erythromycin production by nearly 90 per cent, but vitamin B_{12} production and growth were reduced by only 20 per cent. No direct relationship could thus be demonstrated between the production of erythromycin and of B_{12} (Musílek, 1959).

The addition to submerged culture of arsenite, which inhibits the second step of oxidative decarboxylation of pyruvic acid to acetic acid (*i.e.*, the oxidation of acetaldehyde), allows the accumulation of pyruvic acid and acetaldehyde at the beginning, but the anoxidative process of biosynthesis of acetylmethylcarbinol from these substrates is intensified. The inhibitory effect of arsenite on erythromycin biosynthesis is suppressed to a large degree not only by the addition of sodium acetate and propionate but also by sodium formate. This suggested that formic acid or its metabolically active equivalent (*e.g.*, formaldehyde) is closely connected with the biosynthesis of erythromycin. When washed mycelium was used, the anoxidative biosynthesis of acetylmethylcarbinol from free acetaldehyde took place. Unlike the oxidation of acetaldehyde, the oxidation of succinate was not inhibited by arsenite. These results pointed further to the essentiality of acetic acid as an intermediate of erythromycin biosynthesis (Musílek and Ševčík, 1959).

Chain (1958) postulated the mechanism of the biosynthesis of erythromycin as fol-

lows. According to the structure of its molecule, a propionyl unit is found to repeat itself regularly. The structure of other macrolides suggests that both acetyl and propionyl residues take part in the condensation process. The macrolides were said to be built up by a mechanism similar to that by which the long-chain fatty acids are synthesized, *i.e.*, by condensation of coenzyme-activated acetyl residues or propionyl residues to polyketo acids, which are then reduced.

TETRACYCLINES

S. aureofaciens was found (McCormick *et al.*, 1959) to produce 2 gm of chlortetracycline per liter of media of simple chemical composition. When washed cell inoculum was previously grown in a corn steep medium, the yield increased to 4 gm. With glycerol as a source of carbon and ammonium ion as a source of nitrogen, the other elements required were inorganic, notably sulfur, phosphorus, and chlorine.

Chain (1958) suggested that the position of the oxygen atoms in the tetracycline molecules on alternate carbon atoms indicates some acetate condensation mechanism. Acetate labelled in positions 1 and 2 with radioactive carbon was found to be incorporated readily into the tetracyclines in positions consistent with this type of mechanism of biosynthesis. Chain further believed that the long-chain unsaturated antibiotics of polyene and polyacetylene nature are also built up by acetate condensation. The occurrence of odd-numbered long fatty acid chains may be due to decarboxylation of a dicarboxylic acid.

Darken *et al.* (1960) have shown that in the production of tetracyclines the phosphate-citrate-carbonate ratio is highly critical. As a result of careful study of these interrelationships, yields in shaker flasks were raised from an average of 150 μg per ml to 1350 μg per ml. The high calcium carbonate-citric acid medium permits the calcium ion to act as a sequestering agent for tetracycline. Yields were raised in tank fermentations from 55 μg per ml to 525 μg per ml in the high citric acid-ammonia medium, which permits fermentation in the preferred pH range. The sequestering effect of calcium carbonate becomes apparent as the yields are further raised with this culture to an average of 705 μg per ml.

Each molecule of chlortetracycline contains one chlorine atom. Antibiotic activity can also be produced in a chloride-deficient medium, or one in which chloride utilization is blocked by the presence of low levels of bromide, leading to the production of a new antibiotic, tetracycline (Gourevitch *et al.*, 1955). Controlled biosynthesis is a determining factor in the production of tetracycline, chlortetracycline, or bromtetracycline, depending on the presence or absence of chlorine or bromine in a biologically available form as a precursor. Tetracycline itself is built up by one-carbon transfers from simple starting materials. So far no carbon compound precursors have been found. The biosynthesis of the various chlor- and bromtetracyclines has been studied further by Doerschuk *et al.* (1956).

Miscellaneous Antibiotics

CHLORAMPHENICOL

This antibiotic is made up of two chemical units, *p*-nitrophenylserinol and dichloroacetic acid. By use of a synthetic glycerol lactate medium, it was found that various amino acids and *p*-nitrophenylserinol stimulated chloramphenicol biosynthesis; dichloroacetic acid did not. Both growing and resting cells of *S. venezuelae* are capable of acetylation of *p*-nitrophenylserinol to give N-acetyl-*p*-nitrophenylserinol. However, the latter does not act as a precursor of chloramphenicol, but being itself an antibiotic, it increases the antibiotic potency of the broth (Gottlieb *et al.*, 1956).

NOVOBIOCIN

This antibiotic consists of three distinct moieties: a sugar, substituted coumarin, and substituted benzoic acid (Van Tamelen, 1958). The addition of the last to the medium resulted in an increase in the yield of novobiocin, a fact which suggests the precursory nature of the acid (Jones, 1958).

Conclusions

Examination of the chemical structure of a large number of antibiotic complexes led Abraham and Newton (1958) to conclude that many antibiotics are built up from substances that play an essential metabolic or structural role in the life of microorganisms. Certain types of structure, such as the large lactone or peptide ring, frequently recur. This was believed to be a reflexion of specific types of organization in microbial cells. These workers emphasized that different members of the same type of antibiotic may be produced by such processes as N-, O-, and C-methylation, N-acylation, and the introduction of hydroxyl groups, or by the substitution of one structural unit for another of a similar type. The yield of a given antibiotic can vary enormously with changes in the concentrations of intermediates which accompany changes in cultural conditions; modifications of the enzymatic systems which are a consequence of mutations were also believed to play a role.

Abraham (1959) made a detailed analysis of the phenomenon of biogenesis of antibiotics. According to him, "What we now know, and can reasonably surmise, about the origin of the antibiotics points to the economy with which their novel and sometimes intricate structures are made. Amino acids and acetate, which feature as common structural units, have widespread functions in cellular metabolism. The condensation of acetate, as acetyl coenzyme A, to yield a β-keto acid, and of activated amino acids to form peptide chains, are familiar processes. So, too, is the transfer of a methyl group from S-adenosyl-methionine to nitrogen, oxygen, or carbon in another molecule. D-amino acids are commonly present in the cell walls of bacteria and actinomycetes, though they have not been detected in other forms of life. Large peptide and lactone rings, of a type previously unknown in natural products, have now been identified so often in the structures of antibiotics that their formation would seem to have a broad significance." He concluded that the biosynthesis and mode of action of antibiotics might "be expected to illustrate the diversity rather than the unity of nature." He added, however, that this is only partly true.

Chapter 8

Antimicrobial and Antitumor Activities of Antibiotics

Antibiotics vary greatly in their biological activities, especially in their antimicrobial properties, toxicity to animals, and mode of action upon sensitive organisms. Their practical potentialities, therefore, notably their therapeutic effects in the treatment of human and animal diseases and their growth-promoting action, also vary greatly. Unfortunately, the specific chemical structure of a compound alone is not necessarily indicative of its biological behavior.

It was at first believed that screening methods *in vitro* were not satisfactory for finding new chemotherapeutic substances. Fortunately, this concept was found later to be completely unjustified. Chain and Florey (1944) emphasized that those antibiotics which pass the biological activity and toxicity tests can be expected to be effective as general therapeutic agents. Further studies confirmed these observations, namely, that biological activities of antibiotics *in vivo* are in general parallel to their activities *in vitro*. Usually the only factors controlling their actual practical potentialities are toxicity, selective activity upon various disease-producing organisms, and the extent of such activity.

Antimicrobial Spectra of Antibiotics

Antibiotics are characterized primarily by their selective action upon different microorganisms, or their antibiotic spectra. It was once believed that this property was so characteristic of each substance as actually to identify the antibiotic. However, the ease with which many antibiotics select resistant mutants in bacterial populations, and the frequent variations in the degree of sensitivity among the different strains of the same organism tend to diminish the significance of this property for either the identification of the antibiotic or its proper characterization.

The following types of antimicrobial spectra can be recognized:

1. Antibiotics active mainly against mycobacteria; examples: actithiazic acid and nocardamin.

2. Antibiotics active mainly against gram-positive bacteria; examples: the macrolides such as erythromycin and other antibiotics such as thiostrepton.

3. Antibiotics active against both gram-positive and gram-negative bacteria; examples: streptomycin, neomycin, and the tetracyclines.

4. Antibiotics active against bacteria and fungi; examples: mycothricin and aureothricin.

5. Antibiotics active mainly against fungi; examples: most of the polyenes, such as amphotericin B, and other substances such as cycloheximide.

6. Antibiotics active against intracellular parasites of the lymphogranuloma-psitta-

cosis group. Such antibiotics can be active against other microorganisms; examples: the macrolides and the tetracyclines.

7. Antibiotics active against protozoa. Such antibiotics usually have other types of antimicrobial activity also. They include paromomycin, which is also active against bacteria, and trichomycin, which is also active against fungi.

Substances produced by actinomycetes which are not always strictly antibiotics have other types of biological activity: (1) antiviral substances such as ehrlichin, (2) antitumor substances; these may be antibiotics, such as actinomycin, or have no known antibiotic activity, such as carcinomycin.

The keys and lists in Part B of this book show in more detail the antibiotics belonging to these various groups.

The antibacterial properties of a group of antibiotics produced by actinomycetes are shown in Table 27, in which the sensitivities of different bacteria are summarized according to Turpin and Velu (1957). Streptomycin and neomycin show the greatest activity against the mycobacteria and various gram-negative bacteria, especially the *Escherichia*, *Salmonella*, *Proteus*, and *Pseudomonas* groups; they show little or no activity against the anaerobic clostridia, and limited activity against the neisseriae and certain streptococci. Erythromycins are highly active upon the various cocci and certain gram-positive bacteria, but show only limited action upon gram-negative or-

TABLE 27

Comparative sensitivity of various bacteria to several antibiotics of actinomycetes (Turpin and Velu, 1957)

Results given as μg per ml.

Organism	Streptomycin	Chloramphenicol	Chlortetracycline	Oxytetracycline	Neomycin	Erythromycin
Diplococcus pneumoniae	0.5–50	0.06–12.5	0.03–5	<0.2–<0.4	0.5–150	0.003–0.19
Neisseria gonorrhoeae	1–40	0.078–6.3	0.156–5	0.31–1	R*	0.04–0.50
N. intracellularis	1–40	0.78–6.25	0.20–5	0.20–3.12	4–>300	0.19–3.12
Staphylococcus aureus	0.03–>256	1–50	0.9–125	0.31–2.5	0.1–>300	0.01–62.5
Streptococcus hemolyticus						
Group A	6.2–100	0.3–6	0.8–5	0.156–1.5	0.5–150	0.007–0.62
Group D	0.2–>100	1–15	0.2–25	0.3–3	16–500	0.02–3.12
Streptococcus viridans	3–12	0.6–2.5	0.062–25	<0.03	1–250	0.02–3.12
Bacillus subtilis	0.056–128	1–5	0.06–5	0.15–42	0.1–0.6	0.078
Corynebacterium diphtheriae	0.4–200	0.5–3	0.07	0.15–0.30	0.3–25	0.31–3.12
Mycobacterium tuberculosis	0.1–12.5	6–50			0.1–8	
Various clostridia	R	1.50–>500	0.1–5	0.2–10	30–>150	0.78
Brucella melitensis	0.5–128	1.56–6.25	0.2–5		2.8	
Escherichia coli	0.015–>1000	3–50	0.2–25	0.78–5	0.06–>150	12.5–100
Hemophilus influenzae	0.5–50	0.2–3.5	0.15–5	0.31–2.5	4–>300	0.62–3.12
Klebsiella pneumoniae	0.05–>1000	0.5–25	0.2–50	0.78–3.12	1.5–25	3.12–200
Proteus vulgaris	1–>1000	0.12–>250	0.2–400	0.25–>250	0.25–>150	250
Pseudomonas aeruginosa	0.1–>1000	8–>1000	10–400	5–25	1.6–65	125–>200
Salmonella diverses	0.004–32	0.25–6	0.2–25	2.5–5.5	0.8–83	62.5–>200
Sal. typhosa	0.004–32	0.75–5	0.2–5	<0.3–3.12	0.3–16	62.5
Shigella diverses	0.25–10	0.5–10	0.078–25	0.039–3.12	0.5–10	32

* R = resistant.

ganisms. The tetracyclines and chloramphenicol are active against both gram-positive and gram-negative bacteria, including the clostridia and the brucellae. The last two groups of antibiotics are also active upon the rickettsiae and psittacosis-lymphogranuloma group of intracellular parasitic organisms, but the first group is not.

The phenomena of cross-resistance and strain variation further characterize the above three groups of antibiotics, as will be discussed later (Chapter 10). The literature on the various antibiotics is so extensive that no attempt could be made to cover it in even a limited way. Only a few pertinent references can be presented here on the antimicrobial activities of some of the antibiotics produced by actinomycetes, especially those that have been well recognized and are of established therapeutic value.

Finland and Haight (1953) isolated 500 strains of hemolytic, coagulase-positive cultures of *Staph. aureus* from clinical material and tested them for sensitivity to various antibiotics (Table 28). They observed that with an increase in the use of various antibiotics in clinical practice, there was a significant increase in the proportion of bacterial strains resistant to the antibiotics, notably to chlortetracycline and oxytetracycline. Of the total strains tested, about three fourths were resistant to penicillin, one fourth to chlortetracycline, and one third to oxytetracycline.

Potee *et al.* (1954) made a study of 119 strains of freshly isolated cultures of *Proteus*. They comprised 86 strains of *Pr. mirabilis*, 12 of *Pr. vulgaris*, 15 of *Pr. morganii*, and 6 of *Pr. rettgeri*. The sensitivity of these strains to 10 antibiotics varied greatly, depending on the species, as shown in Tables 29 and 30. They were all found to be invariably resistant to erythromycin, although a few showed some sensitivity. Most of the strains were resistant to streptomycin, especially after the previous use of this

TABLE 28

Distribution of 500 strains of Staph. aureus according to grade of resistance to five antibiotics which have been in common use (Finland and Haight, 1953)

Antibiotic	Grade of resistance*	No. of strains	% of strains
Penicillin	S	124	24.8
	I	11	2.2
	R	365	73.0
Chlortetracycline	S	337	67.4
	I	45	9.0
	R	118	23.6
Oxytetracycline	S	266	53.2
	I	73	14.6
	R	161	32.2
Chloramphenicol	S	6	1.2
	I	482	96.4
	R	12	2.4
Streptomycin	S	99	19.8
	I	351	70.2
	R	50	10.0

* S = sensitive; I = intermediate; R = resistant.

antibiotic. Many strains were relatively sensitive to neomycin, which had not been used previously in these particular cases. The sensitivity to chloramphenicol and the tetracyclines varied greatly among the species and strains. In general, neomycin and chloramphenicol were most active; streptomycin came next; erythromycin and penicillin were least active. Bacitracin and polymyxin showed no activity. Almost all strains of all species were inhibited by neomycin in concentrations of 25 to 100 μg per ml. This antibiotic was slightly more active *in vitro* against most of the strains than were streptomycin and chloramphenicol (Tables 29 and 30). When the above results were compared to the findings obtained in 1949, no definite increase in resistance of *Proteus* to penicillin or to streptomycin was found; there was a slight increase in resistance to chloramphenicol

TABLE 29

Susceptibility of 33 strains of Proteus to different antibiotics (Potee *et al.*, 1954)

Previous antibiotic therapy*	Species and varieties of *Proteus*	Minimal inhibiting concentration, μg/ml						
		Chlortetracycline	Oxytetracycline	Tetracycline	Chloramphenicol	Streptomycin	Neomycin	Erythromycin
T	*vulgaris*	12.5	12.5	25	25	50	50	400
P		12.5	6.3	12.5	200	>400	50	400
P		6.3	25	12.5	200	>400	25	>400
P, Ct		6.3	12.5	6.3	200	>400	50	>400
P, S		3.1	3.1	6.3	12.5	50	50	400
None		6.3	3.1	12.5	200	>400	25	>400
P		6.3	12.5	6.3	200	>400	25	>400
P		12.5	6.3	3.1	200	50	50	400
None		3.1	3.1	3.1	200	>400	50	400
P		3.1	3.1	3.1	100	>400	50	400
None		12.5	12.5	6.3	25	50	25	200
P		12.5	6.3	12.5	100	>400	50	>400
None	*morganii*	6.3	3.1	6.3	100	50	25	>400
P, Ct		>400	>400	>400	200	>400	12.5	>400
Ot		3.1	3.1	6.3	12.5	50	25	>400
T		>400	>400	>400	>400	>400	25	400
P, T, Cm		>400	>400	>400	>400	>400	25	>400
None		12.5	3.1	12.5	12.5	>400	25	>400
None		6.3	6.3	6.3	25	25	12.5	400
P		>400	>400	>400	>400	>400	12.5	>400
P		12.5	12.5	12.5	50	12.5	25	>400
P		12.5	12.5	12.5	25	12.5	25	400
Ot, T, Cm		50	12.5	12.5	200	>400	50	>400
P		100	25	12.5	50	12.5	25	>400
P		25	25	12.5	12.5	12.5	25	>400
P		25	12.5	12.5	25	>400	50	>400
B, Pm		>400	>400	200	100	>400	100	50
P	*rettgeri*	>400	>400	>400	>400	>400	400	50
P, S		>400	>400	>400	400	>400	50	400
P, S		>400	>400	>400	>400	>400	25	400
P, S		12.5	6.3	6.3	100	>400	25	400
P		12.5	6.3	12.5	100	>400	50	>400
P, S, Ct		>400	>400	400	12.5	>400	25	>400

* T = tetracycline; P = penicillin; Ct = chlortetracycline; Ot = oxytetracycline; Cm = chloramphenicol; S = streptomycin; B = bacitracin; Pm = polymyxin.

col and a definite increase in the proportion of strains resistant to chlortetracycline. Treatment of patients with penicillin did not appear to influence the susceptibility to this antibiotic of strains of *Proteus* subsequently isolated from such patients. Streptomycin-resistant strains were more frequent among patients previously treated with this antibiotic than among those who had not received streptomycin. Cultures isolated from patients treated with tetracyclines showed a larger proportion resistant to these three antibiotics.

The susceptibility of *Pseudomonas* cul-

TABLE 30

Comparison of antibiotic susceptibilities of 86 strains of Pr. mirabilis with those of 33 strains of other species of Proteus (Potee *et al.*, 1954)

Antibiotic	Species and strains of *Proteus*	No. of strains inhibited by (μg/ml)								
		3.1	6.3	12.5	25	50	100	200	400	>400
Chlortetracycline	*mirabilis*	0	0	0	0	0	0	2	4	80
	Others*	4	6	10	2	1	1	0	0	9
Oxytetracycline	*mirabilis*	0	0	0	0	0	0	2	13	71
	Others	7	6	8	3	0	0	0	0	9
Tetracycline	*mirabilis*	0	0	0	0	4	17	43	15	7
	Others	3	8	12	1	0	0	1	1	7
Chloramphenicol	*mirabilis*	0	1	2	13	36	33	1	0	0
	Others	0	0	5	5	2	6	9	1	5
Streptomycin	*mirabilis*	0	3	3	13	38	17	1	0	11
	Others	0	0	4	1	6	0	0	0	22
Neomycin	*mirabilis*	0	0	0	12	57	17	0	0	0
	Others	0	0	3	16	12	1	0	1	0
Penicillin	*mirabilis*	0	1	20	25	8	10	11	4	7
	Others	0	0	0	0	0	0	1	0	32
Erythromycin	*mirabilis*	0	0	0	0	0	1	5	16	64
	Others	0	0	0	0	2	0	1	13	17
Bacitracin	*mirabilis*	0	0	0	0	0	0	0	0	86
	Others	0	0	0	0	0	0	0	0	33
Polymyxin	*mirabilis*	0	0	0	0	0	0	0	0	86
	Others	0	0	0	0	0	0	0	0	33

* *Pr. vulgaris*, 12 strains; *Pr. morganii*, 15 strains; and *Pr. rettgeri*, 6 strains.

tures to various antibiotics is shown in Table 31. Polymyxin B was by far the most active agent, nearly all strains being inhibited by 6.3 μg per ml or less. The tetracyclines and neomycin were next in activity. A few strains were sensitive to streptomycin; most of them were moderately sensitive to other antibiotics. Typical strains of *Ps. aeruginosa* were moderately or highly resistant to erythromycin and chloramphenicol, and all strains were resistant to the highest concentrations of bacitracin and penicillin used, namely, 400 μg per ml. The resistance of the strains to any of the antibiotics could not be correlated with the previous history of treatment of the patient with homologous or other antimicrobial agents.

The sensitivity of different pathogenic organisms to streptomycin is illustrated in Table 32.

The sensitivity of different bacteria to different neomycins is shown in Table 33. Neomycin B was most active, whereas

TABLE 31

Comparison of antibiotic sensitivity of three series of strains of Pseudomonas (Wright *et al.*, 1954)

Antibiotic	Series*	No. of strains	Per cent of strains inhibited by (μg/ml)									
			1.6	3.1	6.3	12.5	25	50	100	200	400	>400
Polymyxin	A	25		72	24	4						
	B	32	3	38	59							
	C	110	4	35	60	1						
Streptomycin	A	185		1	2	5	16	28	27	6	2	14
	B	32				3	9	44	19	3	9	13
	C	110		1	1	2	9	20	18	3	3	44
Chloramphenicol	A	27					0	0	0	33	59	4
	B	32									16	84
	C	110					1	1	1	1	8	88
Chlortetracycline	A	115			1	2	2	9	20	49	17	1
	B	32							9	38	16	38
	C	110			1	1	0	3	5	15	6	70
Oxytetracycline	B	32					3	6	78	13		
	C	110			1	1	5	35	41	17		
Tetracycline	B	32						3	75	22		
	C	110				2	0	5	18	50	24	2
Neomycin	B	32					6	41	28	19	6	
	C	110			3	3	3	13	13	37	26	3
Erythromycin	B	32								22	72	6
	C	110			1	2	1	0	10	50	31	5

* Series A = All strains isolated in 1949 and earlier. Series B = Lyophilized strains isolated in 1949, studied in 1954. Series C = Current strains, isolated December 1953 to May 1954.

neamine was least active. There was much variation among the different species and strains.

The increased use of antibiotics in clinical practice leads to an increase in resistance of bacteria. This is well illustrated in Table 34. There is a difference, however, between different organisms. *Klebsiella*, for example, develops greater resistance than *Escherichia*. The correlation between antibiotic sensitivity tests and clinical results was examined by Abboud and Waisbren (1959).

Antibiotics have been grouped on the basis of their antistaphylococcal properties by Waisbren and Strelitzer (1960) (Table 35). A detailed study of the behavior of new antistaphylococcal agents has been made by Garrod and Waterworth (1956).

Considerable work has been done and a most extensive literature has accumulated on the sensitivity of the tuberculosis organism *Mycobacterium tuberculosis*, to various antibiotics and other antimycobacterial agents. Waksman and Lechevalier (1953) have shown that the human and bovine forms of *M. tuberculosis* are highly sensitive to isoniazid, the avian form is less sensitive, the nonpathogenic mycobacteria are still less sensitive, and the *Nocardia* and *Streptomyces* species are fairly resistant. Pollak (1956) reported that atypical acid-fast mycobacteria are sensitive to streptomycin and partially or totally resistant to the synthetic chemotherapeutic agents *p*-aminosalicylic acid and isoniazid.

Microbiostatic *versus* Microbicidal Activity of Antibiotics

Antibiotics can kill microbial cells, or they may simply prevent their multiplication.

TABLE 32

Sensitivity of pathogenic microorganisms to streptomycin in vitro (Youmans and Fisher, 1949)

Sensitive

Actinomycetes	*M. tuberculosis* var. *bovis*
Bacillus anthracis	*Neisseria gonorrhoeae*
Brucella abortus	*N. meningitidis* (*N. intracellularis*)
Br. melitensis	*Pasteurella multocida*
Br. suis	*P. pestis*
Donovania granulomatis	*P. tularensis*
Erysipelothrix rhusiopathiae	*Salmonella* species
Hemophilus influenzae	*Sal. typhosa* (*Eberthella typhosa*)
H. pertussis	*Shigella dysenteriae*
Klebsiella pneumoniae	*Sh. paradysenteriae*
Leptospira icterohaemorrhagiae	*Streptobacillus moniliformis*
Listeria monocytogenes (*Listerella monocytogenes*)	*Veillonella gazogenes*
Mycobacterium tuberculosis var. *hominis*	

Moderately sensitive

Aerobacter aerogenes	*Pr. vulgaris*
Alcaligenes faecalis	*Pseudomonas aeruginosa* (*Bacillus pyocyaneus*)
Corynebacterium diphtheriae	*Rickettsia akari*
Coxiella burnetii (*Rickettsia burneti*)	*R. prowazekii*
Diplococcus pneumoniae	*R. typhi* (*R. mooseri*)
Escherichia coli	*Streptococcus*, α hemolytic
Hemophilus ducreyi	*Streptococcus*, β hemolytic
Malleomyces mallei	*Streptococcus faecalis*
Staphylococcus albus	*Vibrio cholerae* (*V. comma*)
Staph. aureus	
Nocardia asteroides	
Proteus morganii	

Insensitive

Bacteroides fragilis	Psittacosis organism
Bacteroides fundiliformis	*Blastomyces dermatiditis*
Clostridium species	*Candida albicans*
Malleomyces pseudomallei	*Coccidioides immitis*
Rickettsia tsutsugamushi (*Rickettsia orientalis*)	*Cryptococcus neoformans* (*Torula histolytica*)
Feline pneumonitis organism	*Geotrichum* species
Virus of human influenza	*Histoplasma capsulatum*
Lymphogranuloma venereum	*Endamoeba histolytica*
Meningopneumonitis	*Trichomonas vaginalis*
Virus of mumps	*Trypanosoma* species

TABLE 33

Responses of bacteria to neomycin B, neomycin C, and neamine (Sebek, 1958)

Organism	Maximal tolerated concentration		
	Neomycin B	Neomycin C	Neamine
	$\mu g/ml$		
Serratia marcescens	0.8	12.5	1.5
Micrococcus pyogenes var. *albus*	0.8	1.5	6.0
Bacillus brevis	0.8	6.0	6.0
Corynebacterium diphtheroides	1.5	6.0	6.0
Neisseria catarrhalis	1.5	6.0	6.0
Aerobacter aerogenes	1.5	3.0	12.5
Klebsiella pneumoniae	3.0	6.0	12.5
Escherichia coli	3.0	12.5	12.5
Micrococcus pyogenes var. *aureus*	3.0	12.5	25.0
Shigella dysenteriae	6.0	25.0	25.0
Salmonella paratyphi	6.0	25.0	25.0
Proteus vulgaris	6.0	50.0	25.0
Salmonella pullorum	6.0	25.0	50.0
Shigella paradysenteriae var. *sonnei*	12.5	25.0	25.0
Salmonella schöttmuelleri	12.5	50.0	>100.0
Sal. hirschfeldii	12.5	100.0	>100.0
Pseudomonas aeruginosa	12.5	>100.0	>100.0
Streptococcus pyogenes	25.0	25.0	12.5
Streptococcus viridans	25.0	25.0	12.5
Diplococcus pneumoniae	25.0	50.0	50.0
Alcaligenes faecalis	>100.0	>100.0	>100.0
Micrococcus lysodeikticus	1.5	50.0	>100.0
Corynebacterium sp.	1.0	250.0	250.0
Sarcina lutea	1.0	250.0	250.0

In the latter case, growth would take place in the presence of nutrients, after the antibiotic had been washed off the cells.

The microbicidal action of antibiotics

TABLE 34

Number and percentage of coliform bacilli resistant to four antibiotics isolated in two periods (Vaccaro *et al.*, 1956)

Antibiotic	*Escherichia*				*Klebsiella*			
	1951–1952		1955–1956		1951–1952		1955–1956	
	No.	%	No.	%	No.	%	No.	%
Streptomycin	125	45.4	100	64.1	49	50	72	85.7
Chloramphenicol	40	15.5	28	17.9	22	22.4	36	45.2
Chlortetracycline	118	42.5	69	44.2	33	33.6	60	71.4
Oxytetracycline	106	41.7	77	49.3	36	36.7	63	75
Total strains studied	253		156		98		84	

depends on the concentration of the antibiotics which are put in contact with microbial cells and on the amount and nature of nutrients and salts present. Environmental conditions, such as pH, also play an important role in the killing process.

Many antibiotics have strong bactericidal action. The addition of 0.5 mg of actinomycin to a 10-ml suspension of *E. coli* reduced the number of viable cells from 6,400,000 to 493,000; 1 mg of the antibiotic brought about a reduction to 4,800; and 2 mg resulted in complete destruction of all the living cells.

The microbiostatic action of very similar antibiotics can differ. For example, the bacteriostatic action of both mannosidostreptomycin and dihydromannosidostreptomycin is significantly less than that of streptomycin and dihydrostreptomycin for all organisms except *Salmonella typhosa* and *Salmonella schöttmulleri*. These results are similar to those previously reported (Rake *et al.*, 1947).

Similarly, Lechevalier (1960) reported differences in the fungicidal properties of polyenic antifungal antibiotics.

A strong lytic action, similar in some cases to the action of enzymes, has also been indicated for some of the antibiotics. This effect may be a result of the lysis of the cells. Autolysis is usually defined as the destruction of some of the cell constituents by enzymes originating within the cell. The lytic effect is brought about, however, by but few of the antibiotics and does not affect most of the bacteria. The greatest bactericidal action of penicillin, for example, occurs during maximal cell division, when the cells capable of producing lytic agents undergo lysis rapidly. It must therefore be concluded that lysis of the cells follows the killing effect of penicillin.

Interaction among Antibiotics

It has been noted that when antibiotics are used in mixtures, one antibiotic will sometimes repress or boost the antimicrobial action of another.

Jawetz, in a chapter of a monograph on neomycin (Waksman *et al.*, 1958), has pointed out that antibiotics can be divided into two groups on the basis of their behavior when used in mixtures. Group 1 comprises penicillin, streptomycin, bacitracin, and neomycin, and Group 2 is composed of the tetracyclines, chloramphenicol, and erythromycin. Members of Group 1 are never antagonistic to one another but not infrequently are synergistic. Members of

Group 2 are as a rule neither antagonistic nor synergistic to one another. When a member of Group 1 is added to a member of Group 2, the effect is unpredictable and depends on the microorganism.

Various methods of determining synergism and antagonism among antibiotics have been proposed and were reviewed briefly by Chabbert and Patte (1960), in a paper in which they described a method permitting the study of the bactericidal synergistic effect of mixtures of antibiotics.

Actinomycetes have been found to produce mixtures of antibiotics which are synergistic. For example, antibiotics PA 114A and B are more active in combination than when used alone.

Geminimycin is the perfect example of such synergistic antibiotic pairs (Rao *et al.*, 1960). It is formed of two compounds, A and B, which are antibiotically inactive. The mixture A + B is active against gram-positive bacteria.

Antifungal Activities of Antibiotics

It has been pointed out elsewhere that actinomycete antibiotics active upon fungi, beginning with cycloheximide and ending with many of the polyenes, have either no activity at all or only very limited activity upon bacteria. These antibiotics are frequently spoken of as antimycotics. Different fungi, often strains of the same species, differ greatly in their sensitivity to these agents. *Trichophyton mentagrophytes* and *Candida albicans* are commonly used as test organisms, with the agar-cup method. Bergman (1955) suggested use of conidia only, a conidial "bank" being recommended for this purpose. Alcohol is usually used as the solvent, since most of the agents are not soluble in water. Sabouraud's agar is commonly employed.

A comparative study of the effect of nystatin, amphotericin B, and candidin on

TABLE 35

Grouping of antibiotics on the basis of their activity against staphylococci (Waisbren and Strelitzer, 1960)

Drug	Group*	Minimal inhibitory concentration (interpolated from means of inhibitory tube)	Per cent of strains susceptible to 6 μg/ml or less of the agent (Abboud and Waisbren, 1954)
		μg/ml	
Neomycin	1	0.35	100
Kanamycin	1	0.36	100
Paromomycin	1	0.41	100
Novobiocin	1	0.51	96
Vancomycin	2	0.86	100
Ristocetin	2	1.40	100
Nitrofurantoin	3	5.79	84
Oleandomycin	3	5.40	73
Erythromycin	3	5.43	59
Streptomycin	3	7.62	59
Tetracycline	3	7.20	43†
Oxytetracycline	3	8.40	40
Penicillin	4	11.46	47†
Polymyxin B	4	11.10	40
Bacitracin	4	11.70	35
Chlortetracycline	4	14.52	35
Chloramphenicol	4	21.60	15

* Significant differences among members of the groups were determined by analyzing the difference of the means of the number of tube dilutions necessary for inhibition of the 75 strains of staphylococci. The following formula was used:

$$T = \frac{D}{\sqrt{N_1 S_1^2 + N_2 S_2^2}} \cdot \frac{\sqrt{N_1 N_2 (N_1 + N_2 - 2)}}{N_1 + N_2}$$

Antibiotics were considered to be significantly different in activity if the P value of the difference of means was <0.01; *i.e.*, if there was less than one chance in 100 that the means of the number of tubes necessary for inhibition with each antibiotic belonged in the same distribution and were not representatives of different distributions. The tubes were numbered as follows: >minimal inhibitory concentration (>100) = 11; 100 = 10; 50 = 9; 25 = 8; 12 = 7; 6 = 6; 3 = 5; 1.5 = 4; 0.75 = 3; 0.38 = 2; <0.38 = 1.

† This means that a slightly greater percentage of strains may be clinically susceptible to penicillin, but that over-all tetracycline is more active on a weight for weight basis.

experimental moniliasis has recently been carried out by Kosunen (1959).

Antitumor Activities of Antibiotics

The ability of various antibiotics to suppress the development of neoplasms resulted in the isolation of a large number of compounds from cultures of actinomycetes that possess such a property (Reilly, 1953). This was brought out in Chapter 3. Ševčík (1959) divided these compounds into four categories on the basis of their antibiotic and antitumor spectra:

1. Substances active upon tumors, as well as upon bacteria. These include actinoxanthine, azaserine, 6-diazo-5-oxo-L-no-leucine (DON), cellocidin, alazopeptin, netropsin, carzinophilin, aburamycin, actinomycin, ractinomycin, pluramycin, amicetin, gancidin, actinoleukin, griseolutein, levomycin, sulfocidin, puromycin, desertomycin, mitomycin, and others.

2. Substances active upon tumors and upon only one group of bacteria, namely, *M. tuberculosis*. These include toyocamycin and tubercidin.

3. Substances active upon tumors and fungi, but not upon bacteria. These include cycloheximide, hygroscopin, and polyenes.

4. Substances active only upon tumors. These include melanomycin, carzinocidin, carcinomycin, and sarkomycin.

The above groups were further subdivided on the basis of their solubility in water. Ševčík found the third group, comprising the polyene compounds, to be the most widely distributed in nature, although he doubted their practical usefulness. The nucleotide antibiotics (puromycin, amicetin, and carzinophilin A) appeared to be most promising because of their relatively low toxicity. The quinone-type antibiotics (actinomycins, pluramycin, ractinomycin, actinoleukin, and levomycin) were highly toxic.

Oda (1960) also summarized our recent knowledge of antitumor antibiotics. Different experimental tumors in animals are used for screening purposes, such as Yoshida sarcoma, Ehrlich carcinoma, mouse leukemia, and others in Japan; sarcoma 180, carcinoma 755, and mouse leukemia in the United States. Oda emphasized that "the present situation of antitumor antibiotic research seems to be in the night before the discovery of streptomycin and the author wishes to here introduce an outline of research of antitumor antibiotics." As many as 2 per cent of all cultures of actinomycetes isolated from soil possess antitumor activity.

Considerable information has accumulated concerning the mode of action of some of these antibiotics, especially actinomycin, upon the tumor cells. Robineaux *et al.* (1958), for example, have shown that in tissue culture, antimitotic activity of actinomycin C is completely repressed by glutathione; cytostatic activity is not affected, however. They suggested that actinomycin possesses at least two mechanisms: antimitotic and cytocidal.

In speaking of the effect of actinomycin D upon transplantable animal tumors, Sugiura (1960) stated, "The effectiveness, at least temporary, of this antibiotic against human neoplasia (Wilms's tumor, neuroblastoma, rhabdomyosarcoma, lymphosarcoma, Ewing's tumor, and melanoma) affords some hope in the attainment of our goal, the cure of cancer in man."

Various methods for the determination of cytotoxic metabolites formed by microorganisms have been suggested. Perlman *et al.* (1959) tested a number of antibiotics for inhibition of multiplication of an L cell line of mouse fibroblasts and showed that various actinomycins (Fig. 3) were remarkably active.

Antitoxin Activity of Antibiotics

Various antibiotics possess remarkable antitoxin properties. Hinton and Orr (1960), for example, have shown that inhibition of

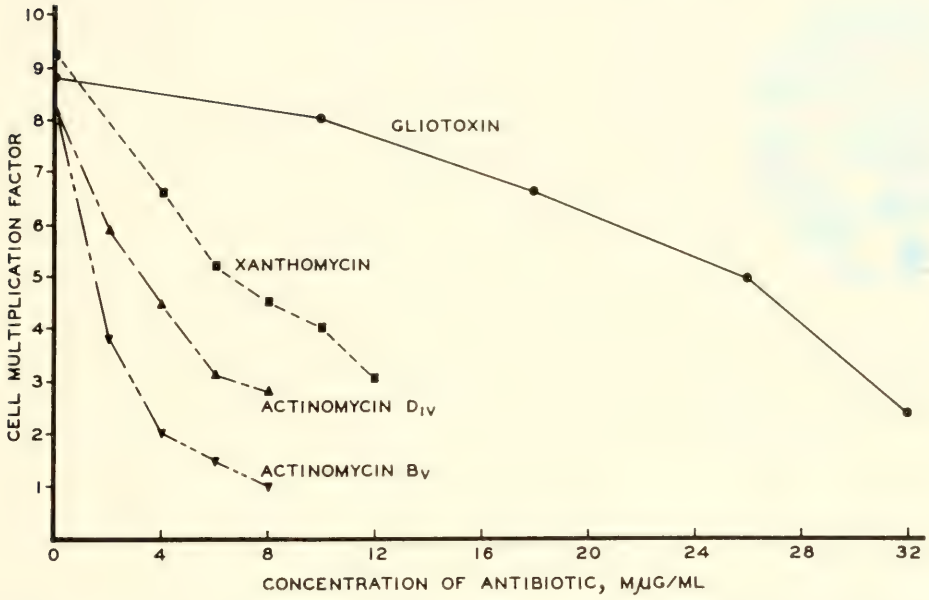


FIGURE 3. Effect of antibiotics on the multiplication of L cells of mouse fibroblasts. (Reproduced from Perlman *et al.* Proc. Soc. Exptl. Biol. Med. **102:** 290–292, 1959.)

toxin production in *Staph. aureus* by chloramphenicol, the tetracyclines, and oleandomycin is directly proportional to inhibition of growth. Streptomycin and bacitracin inhibit toxin production to a degree out of proportion to growth inhibition.

Chemical Structure and Antibiotic Activities

The marked variations in the antimicrobial activities of the antibiotics, or their antimicrobial spectra, present a type of problem that defies any attempt at logical speculation on the possible relation between chemical structure and biological activity. Some antibiotics possess a wide or broad spectrum of antimicrobial activity; others are characterized by a very narrow spectrum of activity.

The following questions logically present themselves in this connection:

Why do some antibiotics act upon bacteria alone, others on fungi alone, and still others upon both bacteria and fungi? Why do some affect the rickettsiae and the other intracellular parasites and not the true viruses?

Why are the gram-positive bacteria far more sensitive to the great majority of antibiotics produced by actinomycetes than are the gram-negative bacteria?

Why do some antibiotics have a marked activity upon acid-fast bacteria, whereas others, even though active upon various gram-positive bacteria, have no effect?

Why do certain closely related groups of bacteria, such as those found among the aerobic spore-formers (*B. subtilis versus B. mycoides*) or among the gram-negative bacteria (*E. coli versus A. aerogenes*), often differ greatly in their sensitivity to certain antibiotics?

Why are some antibiotics active only upon gram-positive bacteria and not upon gram-negative forms, whereas some are active on both gram-positive and gram-negative bacteria?

Why do some bacteria and not others

develop rapid resistance to some antibiotics and not to others, as in the case of staphylococci *versus* streptococci to penicillin? Why do the rates of development of resistance differ for different antibiotics, as in the mechanism of development of resistance among sensitive bacteria to neomycin *versus* streptomycin?

Why do some bacteria produce strains that become nutritionally dependent upon certain antibiotics, such as streptomycin?

Were answers found to these questions, one could go a long way in establishing the correlation between chemical structure and biological activity of the various antibiotics.

Even if they cannot be answered at present, advantage is taken of some of the known properties of the antibiotics in classifying them, utilizing them, and suggesting an interpretation of their possible mode of action. These can be briefly listed as follows:

1. The phenomena of resistance and of sensitivity of microorganisms to various antibiotics permit the recognition of certain close relationships among such antibiotics, if not in their chemical structure, at least in their biological activity. Thus, it is possible to recognize the similarity among antibiotic preparations long before their chemical nature has been established.

2. Although it is now fully recognized that the modes of action of various antibiotics differ, too little is still known about this phase of antibiotic behavior to warrant speculation upon any possible relationships between structure and activity.

3. The toxicity of antibiotics to animal tissues is known to differ greatly. This phenomenon is of importance in any effort to evaluate the practical potentialities of antibiotics in disease control. The reasons for it, however, remain obscure. Neomycin, for example, was shown to have an effect in the treatment of tuberculosis, but it has so far not taken a significant place in the armamentarium of phthisiologists, largely because of its injurious nephrotoxic and ototoxic effects when administered parenterally. Different modes of administration or the supplementation of certain nutritional factors may be the answer for the practical utilization of this antibiotic.

4. Although it has been assumed that activities of antibiotics *in vitro* and *in vivo* are parallel, there are certain instances in which they are not. This is true, for example, of cycloserine, an antibiotic found to be more active against the tubercle bacillus *in vivo* than *in vitro*.

5. Finally, attention must be directed to the facts that actinomycetes also produce growth-promoting substances (B_{12}), and that some antibiotics, in limited concentrations, may also exert a growth-promoting effect upon various forms of life—a property of certain antibiotics that has been taken advantage of in the feeding of poultry, swine, and other animals. These properties tend to complicate further our concept of the chemical structure and biological activity of antibiotics.

The following illustrations will suffice to emphasize that certain changes in the chemical structure of the antibiotic molecule may result in marked changes in its biological activity:

1. When streptomycin is changed chemically to dihydrostreptomycin, whereby the carbonyl group in the central hexose unit is reduced, the characteristic antibacterial properties of the drug are retained, although there is a change in the nature of its potential toxicity. On the other hand, the treatment of streptomycin by such carbonyl reagents as hydroxylamine brings about inactivation of the drug. The replacement of the CH_3 group in the central hexose unit (streptose) by CH_2OH, to give hydroxystreptomycin, seems to increase the toxicity

of the drug without apparently interfering with its activity.

2. Modification of the aromatic aryl, the dichloroacetyl, and the CH_2OH groups in the chloramphenicol molecule results in the complete destruction of its activity.

3. The degradation of chlortetracycline to tetracycline, whereby the chlorine atom in the first ring is replaced by a hydrogen atom, results in a decrease in the antimicrobial activities of the molecule, and is believed to give a less toxic and more stable compound. Dechlorination of chlortetracycline increases its stability markedly.

4. The activity of actithiazic acid against the tuberculosis organism has been related to its thiazolidone structure.

These facts are too limited to justify any broad generalization concerning specific structure and activity of antibiotics. One point is clear, however. The antibiotics of actinomycetes represent such a wide variety of chemical structures and biological activities that we must conclude that we are dealing here with a new field of natural products, varying greatly in chemical composition, in antimicrobial activities, and in other biological properties that render them potentially of great importance to human health and human economy.

Chapter 9

Modes of Action of Antibiotics

Ehrlich defined chemotherapeutics as substances which are bound directly to pathogenic microbial cells, damaging the microbe without affecting the host. At first this concept was not generally accepted. The prevailing idea was that the action of chemotherapeutic agents consisted in stimulating the body defenses against the invading microbes. Later this idea appeared to be supported by the demonstration that prontosil was effective *in vivo* but not *in vitro*. Ehrlich's concept was fully substantiated only when it was established that the active substance in prontosil was sulfanilamide, which was split off in the body, and that this substance was active both *in vitro* and *in vivo*. The action of antibiotics upon pathogenic organisms served to support this idea further, thus contributing materially to the rapid progress in the utilization of antibiotics as chemotherapeutic agents. Some investigators went even further in suggesting that, since the activity of therapeutic agents upon microbes consists largely in their effect upon microbial metabolism, all substances which have such an effect should be considered as antibiotics (Ericsson and Svartz-Malmberg, 1959). Such a concept is scarcely justified, however, since all sorts of complexes, both of natural origin and synthetically produced, have therapeutic potentialities.

We still know relatively little concerning the mechanisms involved in the selective action of antibiotics upon different bacteria and other microorganisms. This phenomenon cannot be correlated with either the morphological or staining properties of the sensitive organisms. True, some antibiotics are active largely upon bacteria and others upon fungi or animal forms, such as amoebae and trypanosomes, but the differences in their action are so marked that no broad generalizations can yet be made.

Most investigators have emphasized the modes of action of clinically useful antibiotics. Some (Lardy *et al.*, 1958) described the action of antibiotics which for various reasons are not useful therapeutically, especially when considered as potential antitumor agents.

Numerous theories have been proposed to explain the modes of action of antibiotics. This action has been attributed to the following phenomena:

1. The antibiotic interferes with microbial cell division, thus preventing further growth of the organism. The cell, unable to divide, gradually dies.

2. The antibiotic interferes with the metabolic processes of the microbial cells by substituting for one of the essential nutrients. A specific inhibitory effect may be exerted by those substances that are structurally related to normal cell metabolites. Such substances are taken up by the cell in competition with normal nutrients. Since they are useless to the cell for further reactions, they block the process of growth.

3. The antibiotic interferes with various enzymatic systems, such as the respiratory mechanism of the microbial cell, especially

the hydrogenase system and the phosphate uptake by the bacteria accompanying glucose oxidation.

4. The antibiotic inhibits cellular oxidations involving nitrogenous compounds.

5. The antibiotic interferes with the production and utilization of a growth factor essential to the cell.

6. The antibiotic combines with the substrate or with one of its constituents, which is thereby rendered inactive for bacterial utilization.

7. The antibiotic favors certain lytic mechanisms in the cell, resulting in destruction of the cell.

8. The antibiotic affects the surface tension of the sensitive organisms, acting as a detergent.

9. It has also been suggested that the activity and specificity of an antibiotic are functions of several factors, such as diffusibility of the antibiotic into the microbial cell, adsorption by various enzyme systems, its reaction with sulfhydryl groups of the enzymes or with other sulfhydryl-containing substances adsorbed by the enzymes.

10. The concentration of the antibiotic and the composition of the medium are highly important in modifying the activity of the antibiotic. Some antibiotics lose considerable bacteriostatic activity when incubated with sterile broth.

The majority of antibiotics exert not only a marked bacteriostatic effect, but also a bactericidal action. This effect is accelerated by an increase in temperature from 4 to 42°C, but is impaired by an increase in acidity of medium between pH 7.0 and 5.0. The rapid drop in the number of bacteria within the first 15 minutes after the application of penicillin was interpreted as indicative of its bactericidal action. Young cells are particularly susceptible, whereas mature cells are neither lysed nor readily killed. The bacteriolytic action of penicillin upon sensitive organisms is greatest at the maximal rate of multiplication.

Host defenses play a significant role in the clinical efficacy of antibiotics. Here too, however, the precise mechanisms still await clarification. It has been reported, for example, that prior treatment of bacteria with penicillin or with streptomycin sensitizes the organisms to phagocytosis (Linz, 1953). The fact that chlortetracycline, oxytetracycline, and chloramphenicol are therapeutically effective at bacteriostatic rather than bactericidal concentrations implicates the host defense mechanisms in clinical medicine. These observations were summarized by Eagle and Saz (1955).

Chain and Florey (1944) divided all the antibiotics into two groups: (1) those that react with the protoplasm of the cell, thus killing both microbial and animal cells, comparable to the action of chemical antiseptics; and (2) those that react with substances having a specific significance in the growth of the bacterial cell. Some of the antibiotics were found to be largely growth inhibiting and have therefore been designated as "bacteriostatics." With the broadening knowledge of antibiotics, this classification became too limited in scope.

It has also been suggested that the chemotherapeutic potentialities of antibiotics may be measured by their effects on bacterial respiration. If the latter is stopped by the addition of an antibiotic in dilution of 1: 1000, the organisms may be said to have been killed; such an antibiotic would therefore be toxic to animal tissues. If, however, the antibiotic produces little or no effect on the respiration of bacteria, the probability was suggested that the antibiotic might possess chemotherapeutic possibilities.

The antibiotics produced by actinomycetes were shown to affect the growth of certain bacteria, such as *B. mycoides*, in the following manner: Cell division is delayed; the cells become elongated, reaching enor-

mous size and assuming most peculiar forms; spore formation is repressed; delayed non-spore-forming variants are produced with a modified type of growth on nutrient media. The cells of bacteria subject to the action of streptothricin are greatly enlarged as a result of incomplete fission.

Action of Specific Antibiotics

Streptomycin

The mode of action of streptomycin upon bacteria has received much consideration. Some of the observations appear to be unrelated. Macheboeuf (1948) reported inhibition by the antibiotics of dephosphorylation of mononucleotides and depolymerization of nucleic acids. Wight and Burk (1951) reported inhibition of oxygen consumption of resting cells of *E. coli* on various substrates and an inhibitory effect of dihydrostreptomycin upon pyruvate fermentation by *E. coli* (Fig. 4). On the other hand, stimulation by streptomycin of oxygen consumption of *E. coli* with some of the same substrates has

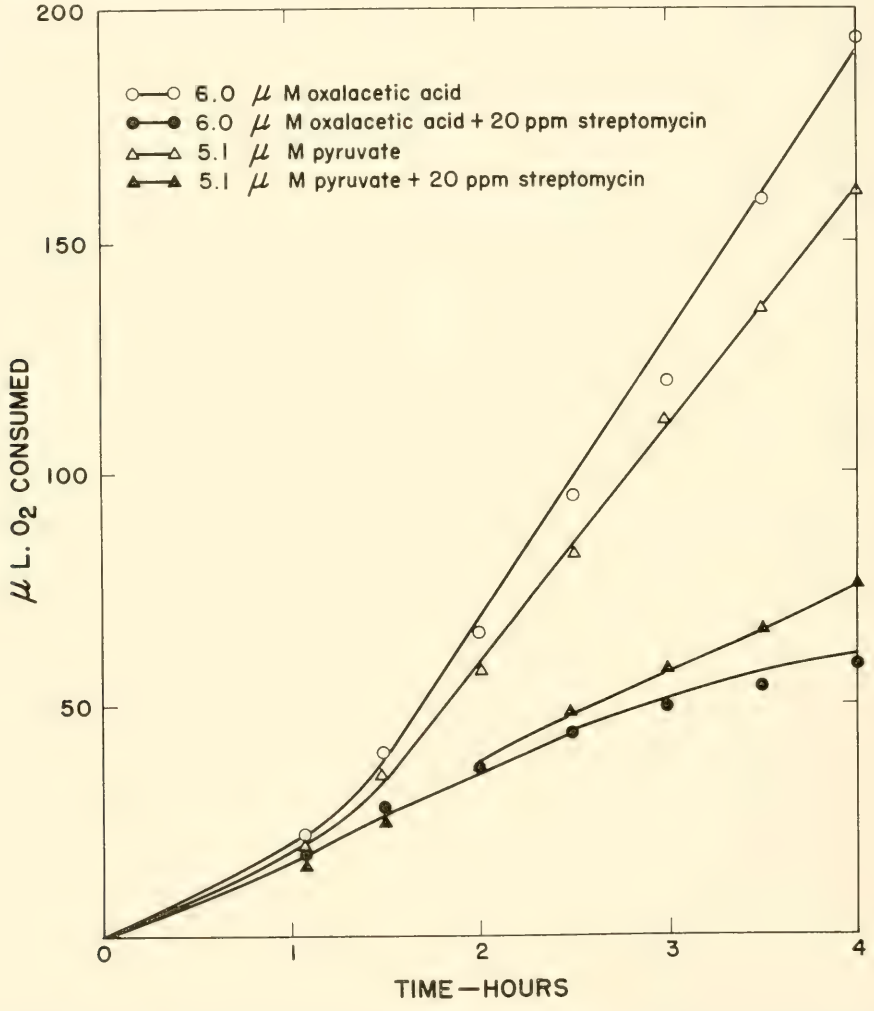


FIGURE 4. Effect of streptomycin on the oxygen consumption involving oxalacetic acid and pyruvic acid. (Reproduced from Wight, K. and Burk, D. Antibiotics & Chemotherapy 1: 380–386, 1951.)

also been suggested (Wasserman, 1953). Zeller (1953) observed an inhibition of diamine oxidase in mycobacteria and ascribed this to the diguanidine residue of the antibiotic.

According to Geiger (1947), the increased ability of *E. coli* cells, first permitted to act on fumarate or other carbon compounds, to oxidize amino acids is nullified by streptomycin. Yoshida and Sevag (1958) suggested that streptomycin interferes with the incorporation of phosphate by *E. coli* cells. The mechanism of action of streptomycin on bacteria was described by Linz (1948) as consisting first of the absorption of streptomycin by the bacterial cells, later followed by the action of the antibiotic on the essential SH groups concentrated in enzymatic complexes.

Lightbown (1954) reported that *Pseudomonas aeruginosa* produced a highly active antagonist of streptomycin and dihydrostreptomycin activity. The antagonist has been identified as a mixture of 4-hydroxyquinoline-N-oxides with alkyl chains of seven, eight, and nine carbons. It inhibits respiration via the cytochrome system (Lightbown and Jackson, 1956). Hancock (1960b) measured the uptake of radioactive streptomycin by cells of various bacteria. When growth is inhibited, the radioactivity taken corresponds to 0.06 μg per mg of cells for *B. megaterium*, 1.5 μg for *Staph. aureus*, and 1.6 μg for *B. subtilis*. The uptake by the first corresponds to about 5×10^4 molecules of streptomycin per cell. In the case of streptomycin-resistant strains, the uptake is then 1 per cent of this amount. When the growth-inhibitory effect of streptomycin (25 μg per ml) on *B. subtilis* is antagonized by 2-heptyl-4-hydroxyquinoline-N-oxide (0.4 μg per ml), the uptake of radioactivity is only about 25 per cent of that in the absence of the antagonist. According to Hancock, "The uptake of streptomycin into the cell is associated with aerobic respiratory processes and is reduced when these are depressed."

Umbreit (1949) and Oginsky (1953) suggested that streptomycin inhibits terminal oxidation in sensitive organisms by inhibiting the condensation of pyruvate and oxalacetate to 2-phospho-4-carboxyadipic acid. This concept has been questioned by Paine and Clark (1954), who reported that a strain of *Staph. aureus*, which under anaerobic conditions simply reduced pyruvate to lactate, was readily killed by the antibiotic. It has also been observed that the antibiotic may inhibit, stimulate, or have no effect on oxygen uptake, depending on the carbon source on which the organism was grown. The killing action of streptomycin was correlated with the metabolic activity of the organism but not with its respiration. The role of streptomycin in the inhibition of synthesis of enzymes responsible for continued oxidation in the cells was also suggested.

Katagiri *et al.* (1960b) could not demonstrate any inhibiting effect of dihydrostreptomycin on the oxalacetate-pyruvate condensation reaction in *E. coli*, nor was the anaerobic production of lactate from glucose inhibited. Anaerobic fermentation of pyruvate by cells grown in a casein hydrolysate medium was strongly inhibited by dihydrostreptomycin; the inhibiting effect of dihydrostreptomycin on the phosphoroclastic split of pyruvate into acetate and formate could also be demonstrated.

However, although the oxidation of pyruvate, acetate, and dicarboxylates by washed cells of *E. coli* was affected only slightly by dihydrostreptomycin or chloramphenicol, the oxidation of these carbon compounds was very sensitive to oxytetracycline. Dihydrostreptomycin showed an accelerating effect on aerobic decomposition of α-ketoglutarate in the presence of appropriate carbon and nitrogen sources by washed, dried, or dry ice-treated cells of *E. coli* or

Ps. fluorescens; no accelerating effect of chloramphenicol was observed (Katagiri *et al.*, 1960a).

Evidence has recently been presented (Anand and Davis, 1960) which indicates the possibility that streptomycin is lethal to sensitive cells because of interference with cell membrane synthesis. This results in drastic changes in the selective permeability of the membrane, with attendant leakage of important constituents such as nucleotides from the cell to the outside. Inward permeability is also affected. Here again, however, one must be absolutely certain that the effects observed are primary effects of the antibiotic and not due to defects associated with dead and dying cells. Hurwitz and Rosano (1960), basing their results upon the observation that chloramphenicol inhibits killing by streptomycin when both drugs are added simultaneously, suggested that a streptomycin-induced specific protein synthesis precedes killing of the cells by streptomycin. The reverse of this is that cells lacking the potential for this induced protein synthesis cannot be killed by this antibiotic. The latter type cells presumably would include both host cells and streptomycin-resistant bacterial mutants.

By using radioactive amino acids for the aerobic growth of *M. tuberculosis* (BCG), Stachiewicz and Quastel (1959) demonstrated that glycine and serine showed distribution of radioactivity in a large number of amino acids of the microbial protein; on the other hand, the use of radioactive alanine and valine resulted in a protein in which only these two amino acids were radioactive. The effect of dihydrostreptomycin, in concentrations at which it exerts inhibitive effects, upon the aerobic growth of *M. tuberculosis* (BCG) consists in the inhibition of protein synthesis. No effect was obtained on labelled amino acid incorporation in *E. coli*, but there was an effect in *M. phlei* (see also Erdös and Ullmann, 1959).

According to Erdos *et al.* (1960), streptomycin inhibits the incorporation of tyrosine into proteins of a sensitive strain of a saprophytic *Mycobacterium*; on the other hand, the incorporation of tyrosine into resistant strains, as well as in a somewhat dependent strain, was increased by the antibiotic. RNA synthesis, either in resistant or sensitive strains, was not influenced by streptomycin. The antibiotic was said to inhibit transport of amino acids from RNA to proteins.

According to Shaw *et al.* (1960), mannosidostreptomycinase activity was inhibited by the addition of Fe^{++}, Ni^{++}, Zn^{++}, or Cr^{6+} at the beginning of the fermentation. The possible inhibition of mannosidostreptomycinase synthesis by Fe^{++} was suggested. Ca^{++} added after 144 hours to fermentation broths containing 50 to 60 ppm of Fe^{++} reversed the inhibition of enzyme activity because of the presence of the ferrous ion. Streptomycin production was reduced markedly by Ni^{++} or Cu^{++}, but was reduced only slightly in the presence of both Ni^{++} and Fe^{++}. Further information on streptomycinase is given by Sakakibara (1951) and on the microbial degradation of streptomycin, by Pramer and Starkey (1951) and Klein and Pramer (1960).

Henry and Hobby (1949) reported that streptomycin activity varies directly with concentration of the antibiotic, and inversely with hydrogen ion concentration. Streptomycin is both bacteriostatic and bactericidal, depending on various factors. The bacteriostatic action of streptomycin appears after a certain lag period. This action is antagonized by most inorganic and organic salts and by many sulfhydryl compounds. Streptomycin was believed to inhibit the metabolism of carbohydrates, ribonucleic acid, benzoic acid, and amino acids. It was suggested that the process of cell division or the synthesis of protoplasm is blocked by interference by streptomycin with one or more enzyme systems essential to these functions. Han-

cock (1960a) examined in detail the factors involved in bactericidal action of streptomycin upon *Staph. aureus*.

The ability of streptomycin to destroy chloroplasts was first demonstrated by von Euler (1947). Provasoli *et al.* (1951) have shown that bleached races of the flagellate *Euglena gracilis* can thus be obtained. In the absence of an added energy supply, streptomycin also acts as an inhibitor of biosynthesis by protoplast lysates and ghosts of *E. coli* (Reiner *et al.*, 1958).

The antibacterial activity of streptomycin can be largely or completely neutralized or antagonized by an anaerobic environment and by various chemical agents. These include glucose and certain other sugars, certain sulfhydryl compounds, and ketone reagents. The effect of cysteine, of cevitamic acid, and of ketone reagents in inhibiting streptomycin activity may be associated with the blocking of the active grouping in the molecule of streptomycin.

Streptomycin is adsorbed on the bacterial surface, resulting in a reduction of the net negative charge, a change which affects the electrophoretic mobility of some of the cells (McQuillen, 1951). The effect of streptomycin on the intermediary carbohydrate metabolism, especially to the acetate or pyruvate stage, of the bacteria and on amino acid utilization has been variously postulated (DiMarco, 1958). The bacterial cell tries to escape the antibacterial action of the antibiotic, which was believed to be due to interference with the synthesis of cell-wall material, by the synthesis of a different kind of cell-wall substance. Streptomycin-resistant mutants of bacteria may show new deficient characters (Kohiyama and Ikeda, 1960).

Streptothricin

Streptothricin and streptomycin are both active against gram-positive and gram-negative bacteria, but they differ in their antibiotic spectra and in their toxicity to animals, the first exerting a delayed toxic action. They are soluble in water but insoluble in alcohol and other organic solvents. Both have an optimal reaction at pH 8.0, and both are repressed by glucose and by acid salts. They are both stable compounds and are highly resistant to the action of microorganisms. However, the two substances can be differentiated in their relation to cysteine. Streptomycin becomes inactivated by the addition of 3 to 5 mg of this compound to 100 μg of the antibiotic, whereas streptothricin is not affected by cysteine. Streptothricin is also active upon fungi, but streptomycin is not.

Chloramphenicol

Chloramphenicol is structurally related to *p*-nitrophenylserinol. Numerous attempts were therefore made to reverse the bacteriostatic activity of this antibiotic by aromatic amino acids. Woolley (1950) reported that the growth-inhibitory activity of chloramphenicol at a concentration of 1 μg per ml on *E. coli* was completely reversed by the addition of phenylalanine to the medium at a concentration of 500 μg per ml. No reversal of activity was obtained, however, when the chloramphenicol concentration was greater than 2 μg per ml. Similar results were obtained with tyrosine and tryptophan. With *Lactobacillus casei*, only phenylalanine was effective in reversal and, as with *E. coli*, reversal occurred only within a narrow range of concentrations of the antibiotic. Mentzer *et al.* (1950) reported the antagonistic effects of glycine upon the inhibition of *E. coli* by chloramphenicol; aspartic acid and serine also showed some antagonistic potentialities; tryptophan was without effect. Truhaut *et al.* (1951) reported that chloramphenicol inhibited both synthesis and breakdown of tryptophan or its precursors (anthranilic acid, indol) by *Salmonella typhosa*.

The most dramatic effects of chloramphenicol have been reported on protein synthesis and in both bacterial and mammalian tissues. Earlier work (Hahn and Wisseman, 1951; Saz and Marmur, 1953) had shown that the antibiotic inhibited the synthesis of induced enzymes mediating the oxidation of lactose and the phosphorylation of gluconic acid in *E. coli*. No effect of the antibiotic was observed in cells previously adapted to lactose and gluconate oxidation. Gale and Folkes (1953) and Wisseman *et al.* (1954) found that chloramphenicol, at growth-inhibitory concentrations, specifically and immediately inhibited protein synthesis in bacterial cells, whereas formation of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and polysaccharide was inhibited, if at all, only at much higher levels of the antibiotic.

Since these original observations were made, numerous confirmatory reports have followed. There seems little doubt that, upon addition to bacterial cultures, chloramphenicol abruptly inhibits protein synthesis and that presumably this inhibition accounts for its antibiotic activity. Hopkins (1959), for example, demonstrated that chloramphenicol inhibits the incorporation of amino acids into protein of calf thymus nuclei but has no effect on the uptake of leucine by nuclear RNA (Fig. 5). It must be noted, however, that the precise locus of chloramphenicol inhibition of protein synthesis remains to be delineated.

According to Brock (1961), chloramphenicol antagonizes the action of antibiotics which act on growing cells, such as penicillin and streptomycin; however, it exerts an additive effect upon antibiotics which also inhibit protein synthesis, such as the tetracyclines and erythromycin. Chloramphenicol inhibits the incorporation of radioactive amino acids into protein. It does not inhibit the activation of amino acids or transfer of amino acids to soluble RNA, but it prevents some step in their transfer from soluble RNA to protein. A similar behavior of the tetra-

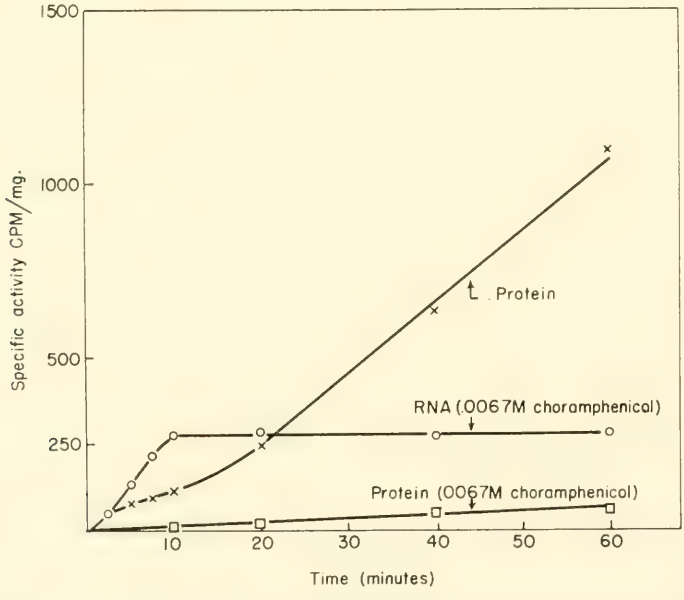


FIGURE 5. Effect of chloramphenicol upon the uptake of leucine-1-C^{14} (5.3 μc per μmole) into ribonucleic acid and protein of calf thymus nuclei. (Reproduced from Hopkins, J. W. Proc. Natl. Acad. Sci. U.S. 45: 1461–1470, 1959.)

cyclines, erythromycin, and puromycin explains the above additive effect.

According to Korotajev (1959), chloramphenicol does not influence the aerobic oxidation of pyruvic acid by resting cells of *E. coli* but inhibits anaerobic pyruvate metabolism. Pyruvate consumption by resting cells of *Shigella flexneri* is inhibited under aerobic conditions and under conditions of limited oxygen supply. Schneierson *et al.* (1960) emphasized that chloramphenicol, in concentrations that fail to inhibit growth, is capable of depressing pigment synthesis by *Pseudomonas aeruginosa*. The antibiotic exerts its action by interfering with biosynthesis of the pigment by the organism, no reducing effect upon pigment already formed being demonstrated. A single exposure to chloramphenicol resulted in a complete and permanent loss of the ability to produce the pigment in three of the four strains tested.

Tetracyclines

It is generally assumed that chlortetracycline, oxytetracycline, and tetracycline, because of their close chemical relationship, have similar, if not identical, modes of action (Hahn, 1958). Tetracycline is the parent compound, chlortetracycline has a chlorine atom in the unsaturated (D) ring, and oxytetracycline instead of a hydrogen has a hydroxyl group in the B ring. The antibiotic spectra of all three are similar (Love *et al.*, 1954), and mutual cross-resistance has been found (Wright and Finland, 1954). There are, however, reports of qualitative as well as quantitative differences in their inhibitory effects, and therefore the possibility must be entertained that differences exist in their modes of action. Guillaume and Osteux (1959) have shown that chlortetracycline inhibits two different enzymatic systems in *Proteus mirabilis*, that of oxidation of glucose, pyruvic acid, and acetate, and that of the citric acid cycle.

The tetracyclines affect oxidation and fermentation in susceptible bacteria; inhibition of protein synthesis has been reported; and finally, by virtue of the strong chelating properties of all three antibiotics, interference with various cellular and enzymatic processes has been suggested as a possible mechanism of inhibition.

The inhibitory activities of the tetracyclines on various oxidative properties of whole bacteria have been noted. In the interpretation of these data, the difficulty of distinguishing between primary action and secondary effects on dead or dying cells must again be considered. For example, it has been reported (McCullough and Beal, 1952) that chlortetracycline at concentrations of 250 to 500 μg per ml inhibits oxidation of glucose, pyruvate, fructose, xylose, and trehalose by *Brucella* (Fig. 6). Oxidation of tricarboxylic acid cycle intermediates by *E. coli*, *Ps. aeruginosa*, and *Pr. vulgaris* was similarly inhibited by high concentrations of chlortetracycline. Oxytetracycline in analogous concentrations was also found to inhibit these oxidations (Wong *et al.*, 1953).

The tetracyclines were also found to cause serious derangement of cellular processes leading to protein synthesis and nucleic acid formation.

According to Bernheim and De Turk (1952), chloramphenicol, streptomycin, and the tetracyclines inhibit the oxidation and to a lesser extent the deamination of phenylalanine, tyrosine, and phenylserine by a strain of *Ps. aeruginosa*. They also inhibit the oxidation of succinate and certain other dicarboxylic acids. It was suggested that these antibiotics interfere with the formation of compounds which may be necessary for the assimilation of ammonia or, in the case of phenylalanine, for its oxidation. Bernheim (1954) has further shown that *Ps. aeruginosa* on contact with succinate produces a cell constituent which can be utilized for the synthesis of an enzyme that oxidizes benzoic acid. The above antibiotics inhibit

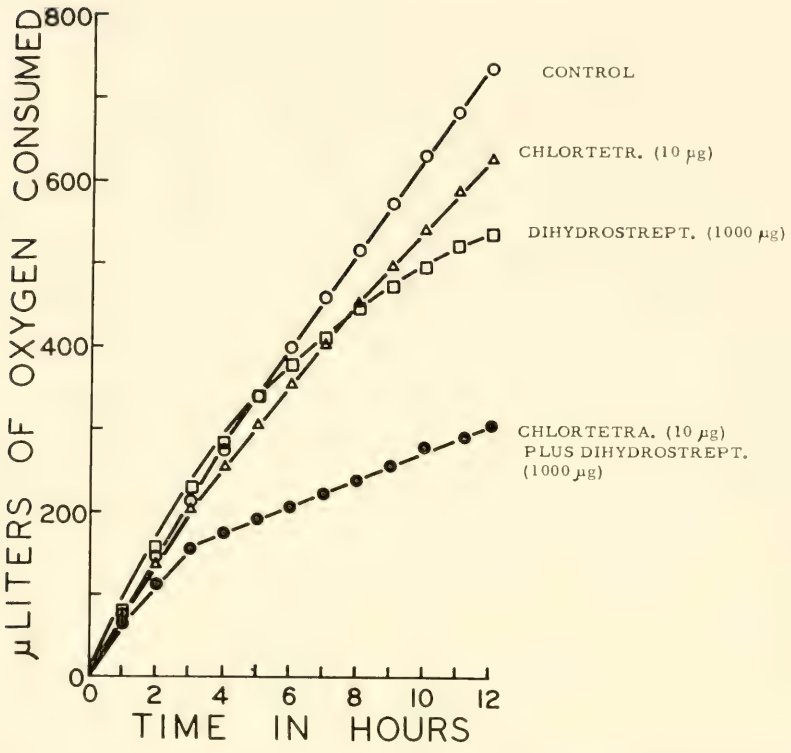


FIGURE 6. Influence of time on the effect of oxygen uptake by *Br. melitensis* by chlortetracycline, dihydrostreptomycin, and chlortetracycline plus dihydrostreptomycin. Glucose added at zero hour. (Antibiotics in presence of cells for 1 hour at room temperature and 35 minutes at 37°C before addition of glucose.) Endogenous respiration values subtracted. (Reproduced from McCullough, N. B. and Beal, G. A. J. Infectious Diseases **90:** 196–204, 1952.)

the formation and utilization of the particular constituent.

In *Staph. aureus*, protein synthesis, as measured by the incorporation of isotopically labelled glutamate, was inhibited by concentrations (0.2 to 0.4 μg per ml) of chlortetracycline and oxytetracycline which were lower than the growth inhibitory levels (0.5 to 1.0 μg per ml). A concentration of 50 to 500 μg per ml was required for inhibition of nucleic acid synthesis, free glutamate incorporation, or glucose fermentation (Gale and Folkes, 1953). Glutathione synthesis by suspensions of *Proteus* was uninhibited even by high concentrations of oxytetracycline and chlortetracycline (Samuels, 1953).

Chlortetracycline in low concentrations inhibited the synthesis of RNA and DNA by *Lactobacillus casei*, and folic acid and vitamin B_{12} were reported to prevent this inhibition (Rege and Sreenivasan, 1954).

Reports have indicated that the tetracyclines inhibit various enzymatic reactions and growth of sensitive organisms by interfering with inorganic ion metabolism.

A highly purified nitroreductase isolated from a chlortetracycline-sensitive *E. coli* was found to be markedly sensitive to precisely those concentrations of the antibiotic which were growth inhibitory. Resolution of the enzyme complex indicated that the reductase was a manganoflavoprotein and that chlortetracycline inhibited the nitroreductase, presumably as a result of its ca-

pacity to chelate or otherwise bind the functional Mn. The locus of inhibition was shown to be at the level of the reoxidation of reduced flavin mononucleotide. It was further observed that the nitroreductase was indeed a portion of the electron transport system of the cell. Thus, inhibition of this aspect of the metabolism of the cell could conceivably account for the antibiotic properties of chlortetracycline. It should be noted that the system described was 100-fold more sensitive to chlortetracycline than to oxytetracycline and tetracycline. It is of interest that a similar system isolated from a chlortetracycline-resistant mutant derived by serial passage from the parent sensitive strain was resistant to chlortetracycline. The presence of firmly bound metal was postulated, and thus the resistant extract successfully competed with the chlortetracycline for essential cation (Saz *et al.*, 1956; Saz and Martinez, 1958, 1960).

Weinberg (1954) investigated the effects of cations on oxytetracycline inhibition of *Ps. aeruginosa* and other cells. The various cations had profound effects on the toxicity of the antibiotic for the cells. Some metals were antagonistic to oxytetracycline, while others potentiated the effects. Rokos *et al.* (1958) have shown that the inhibiting effect of sodium citrate on the action of chlortetracycline on lipase and D-amylase can be explained by the removal of the calcium ion from the system.

The additive effects of chloramphenicol and tetracyclines were discussed by Gale and Folkes (1953) and Ciak and Hahn (1958).

Erythromycin

Erythromycin is active largely upon gram-positive cocci and upon rickettsial organisms. It is either bacteriostatic or bactericidal, depending on the sensitivity of the organism and concentration of the antibiotic. It is active upon multiplying cells but not upon fully grown ones (Haight and Finland, 1952).

Neomycin, Kanamycin, and Other Members of the Neomycin Complex

Gale (1952) made a comparative study of the effect of neomycin and various other antibiotics on amino acid assimilation by *Staph. aureus*. The accumulation of lysine and glutamic acid within the cells was not affected. Protein synthesis was interfered with, however, but not nucleic acid synthesis. The action of neomycin was similar, in this system, to that of chloramphenicol.

According to Tsukamura (1960), kanamycin inhibits the incorporation of P^{32} into the nucleic acid and protein fractions and the incorporation of S^{35} into the trichloroacetic acid-soluble and protein (trichloroacetic acid-insoluble) fractions of the parent sensitive strain of *Mycobacterium avium*, but not of the kanamycin-resistant strain. The ratio of RNA to DNA is higher in the kanamycin-resistant strain than in the parent sensitive strain.

Novobiocin

The effect of novobiocin on the turbidity and viable cells of a growing culture of *E. coli* is shown in Fig. 7. In the case of *Staph. aureus*, it causes the accumulation of N-acetylamino sugar. The fact that the L-isomer exhibits a totally different kind of antibacterial activity, showing a different antibiotic spectrum and a different dose response correlation, was interpreted by Hahn (1958) as illustrating the problematic nature of structure-activity relationship.

Puromycin

Puromycin was found (Creaser, 1955) to be an inhibitor of induced enzyme (β-galactosidase) synthesis in *Staph. aureus*. Chemically, this antibiotic consists of an aminonucleoside linked to an amino acid.

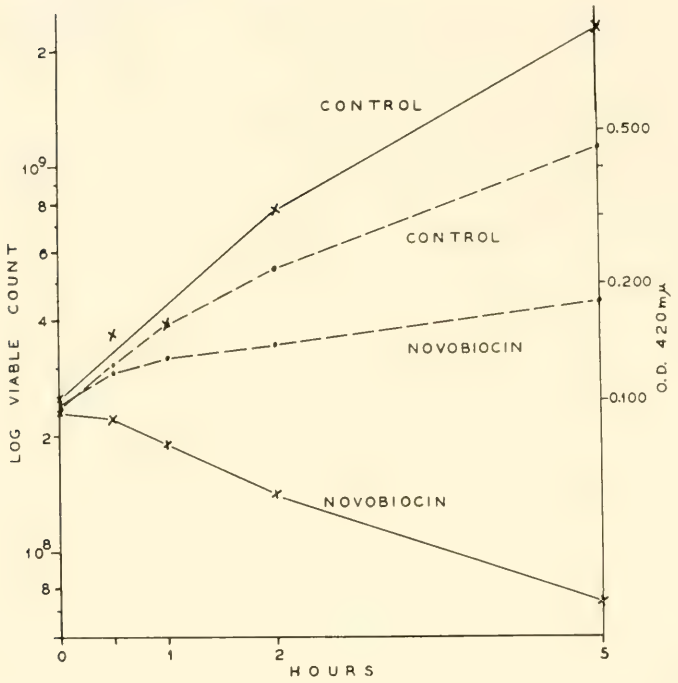


FIGURE 7. Effect of novobiocin (1 mg per ml) on turbidity and viable count of growing cells of *E. coli*; —— = viable count; – – – = turbidity. (Reproduced from Brock, T. D. and Brock, M. L. Arch. Biochem. Biophys. **85**: 176–185, 1959.)

Actinomycin

Actinomycin is a bacteriostatic agent, active primarily upon gram-positive bacteria and to a lesser degree upon gram-negative bacteria. It is also active upon certain neoplasms. It is extremely toxic to animals, a factor which limits its utilization in the therapy of infectious diseases and certain forms of cancer. One milligram of actinomycin given to mice, rats, or rabbits intravenously, intraperitoneally, subcutaneously, or orally is lethal for 1 kg weight of the animals. Doses as small as 50 μg per kg injected intraperitoneally daily for 6 days cause death accompanied by severe gross pathological changes, notably a marked shrinkage of the spleen. Actinomycin is rapidly excreted from the body.

Kawamata and Imanishi (1960) suggested that the carcinogenic effect of actinomycin may be due to its interaction with deoxyribonucleic acid.

Foley (1955) found that the action of actinomycin D upon bacteria consists in interference with pantothenate utilization. This phenomenon could not be confirmed by Slotnick (1957) for *B. subtilis*. Slotnick (1960) demonstrated that actinomycin D suppresses the assimilation of ammonia by *B. subtilis* and inhibits completely the formation of certain inducible enzymes. He concluded that this antibiotic interferes in some reactions leading to protein synthesis.

Kirk (1960) studied the metabolic reaction between actinomycin D and DNA and found that the antibiotic has no significant inhibitory effect on the polynucleotide phosphorylase of *Staph. aureus*, that it inhibits the incorporation of radioactivity into the $HClO_4$-insoluble fraction when P^{32}-deoxyadenosine triphosphate is incubated with a crude preparation of the DNA "polymerase" enzyme isolated from *Escherichia coli*, and that it inhibits the transformation of

H. influenzae from streptomycin sensitivity to streptomycin resistance when it is added to high concentrations of transforming DNA from a resistant strain.

Kersten (1961) recently reported on the inhibition of the growth inhibiting effect of actinomycin upon *Neurospora crassa* and *Streptococcus faecalis* by DNA, RNA, and some of their degradation products (purine moieties). The binding of actinomycin and nucleic acids was demonstrated by changes in the absorption of spectra of actinomycin.

The effect of actinomycin upon the anaerobic carbohydrate metabolism of *Candida albicans* has been studied by Präve (1959).

Azaserine and DON

Azaserine (*o*-diazoacetyl-L-serine) and DON (6-diazo-5-oxo-L-norleucine) inhibit the growth of *E. coli* in a synthetic medium. They interfere with the incorporation of glycine and formate, but not of adenine, into nucleic acids. According to Levenberg *et al.* (1957), they interfere with the biosynthesis of inosinic acid, behaving as competitive inhibitors of glutamine. The action of DON upon the growth of *E. coli*, unlike that of azaserine, is not antagonized by adenine, guanine, hypoxanthine, and the corresponding nucleosides (Maxwell and Nickel, 1957).

Chain (1958) also demonstrated that azaserine interferes with the synthesis of the purine ring system, thus affecting the formation of nucleotide. In the synthesis of inosinic acid by cell-free pigeon liver extracts, azaserine inhibits the formation of formylglycinamide-ribotide and glutamine (in the presence of adenosine triphosphate), apparently acting as a specific antimetabolite to glutamine, to which it is structurally related.

The amoebicidal action of azaserine was investigated by Nakamura (1956).

Polyenes

Among the antifungal agents, nystatin, a polyene antibiotic, has received the greatest attention. This is a tetraene compound with a diene unit, a carbonyl, and a primary amino group. It inhibits the growth of most fungi, in concentration of 1 to 10 μg per ml, but has no effect upon actinomycetes, bacteria, and rickettsiae. It is more effective upon the mycelium than upon the spores of the fungi. It is primarily fungicidal and its action is irreversible. According to Lampen *et al.* (1957), nystatin inhibits the endogenous respiration and the aerobic and anaerobic utilization of glucose and certain other carbon sources by yeasts and other fungi. Low levels of the antibiotic show increased oxygen consumption; high levels show an initial stimulation, followed by abrupt cessation of metabolism, when the cells are no longer viable. Other polyenes (amphotericin) inhibit glycolysis. Nonpolyene antifungal agents (cycloheximide) have no such effect. The conclusion was reached that nystatin blocks a reaction of general metabolic significance to fungi. Further information on the mode of action of nystatin is found in the work of Sutton *et al.* (1960), Marini *et al.* (1960), Tape *et al.* (1960), and Horvath and Szentirmai (1960).

Drouhet *et al.* (1960) studied the effect of amphotericin B on the growing phase of yeasts. It produces an inhibition of the synthesis of proteins, ribonucleic acid, carbohydrate, and polyphosphate reserves. The disturbance of phosphorus metabolism is related to the stimulation of endogenous or exogenous oxidations. The action of other polyenes on respiration is distinct from that of other antifungal antibiotics such as cycloheximide. Amphotericin B produces an increase in O_2 uptake by resting or growing cells of *C. albicans* in the presence or the absence of carbon substrate. This effect is no longer observed on yeasts which have been washed after contact with the antibiotic. It was suggested that the washing eliminates the products responsible for the increase in O_2 uptake, products released by alteration of cell permeability. The mode of action of

other polyenes was studied by Henis and Grossowicz (1960).

Other Antibiotics

According to Aizawa (1955), both the respiration and the adaptive oxidation of mannose and galactose by *Candida albicans* are inhibited by aureothricin, candimycin, eurocidin, and trichomycin; they are not affected, however, by penicillin G, chlortetracycline, oxytetracycline, chloramphenicol, or dihydrostreptomycin.

Abraham (1959) defined some of the main features of bacterial metabolism that are open to selective attack by antibiotics. The formation of the cell walls in bacteria and actinomycetes differs from that of other kinds of cells, although the precise nature of the reactions that are inhibited has yet to be elucidated. The same is true of certain highly specific properties of the bacterial cell which make them sensitive or resistant to a given antibiotic.

Dependence of Microorganisms upon Specific Antibiotics

Among the phenomena related to the antibacterial properties of streptomycin, the development of resistance and of dependence is of particular interest. The problems related to resistance will be considered in detail in Chapter 10. The problem of dependence was first observed by Miller and Bohnhoff (1950). Each of 18 strains of meningococci yielded two variants. One variant, designated as A, grew in large yellowish colonies on streptomycin-free and streptomycin-containing media; it retained the original virulence for mice. The other, B, appeared in greatest numbers in concentrations of 100 and 400 mg per ml of streptomycin. Its colonies varied in size and color, depending upon the concentration of the antibiotic in which they were developed, and were dependent on the presence of streptomycin for multiplication. This dependence was demonstrable not only *in vitro* but also *in vivo*, since the organism exhibited no virulence for mice unless streptomycin was administered to the animals after infection. Both variants retained the characteristic sugar fermentations and type specificity of the parent strain.

The production of streptomycin-dependent strains has also been reported for a number of other bacteria, including *E. coli*, *Ps. aeruginosa*, *B. subtilis*, *Staph. aureus*, and *M. tuberculosis*.

In a study on the distribution of dependent cells of *E. coli* in a broth culture of this organism, Iverson and Waksman (1948) found that one dependent cell was present among each 1.5 billion normal sensitive cells. Streptomycin, and not any accompanying impurity, was required for growth of the dependent organisms. Mannosidostreptomycin and dihydrostreptomycin were also effective in favoring growth of dependent strains; but streptomycin that had been inactivated by cysteine and hydroxylamine was ineffective, as were streptidine and streptamine.

Newcombe and Nyholm (1950) have shown that streptomycin-dependent forms of *E. coli* differ among themselves not only in the degree of their dependence but also with regard to other compounds that have the capacity to replace streptomycin. Dependent strains give double mutants, arising from a second mutation at the original locus, thus forming a continuous series with respect to degree of resistance.

Szybalski and Cocito-Vandermeulen (1958) identified among the streptomycin-dependent mutants of *E. coli* four nutritional groups: (1) Growth is supported solely by streptomycin, or by its dihydro, hydroxy, and desoxydihydro derivatives. (2) Catenulin and neamine, the neomycin-related antibiotics, can substitute for streptomycin. (3) Streptobiosamine, a streptomycin deg-

radation product, forms a substitute for streptomycin. (4) A group similar in properties to the first, but showing restricted growth on appropriate mixtures of catenulin and streptidine or streptobiosamine; it produced frequent mutants which belonged to the second class. Other substances related to streptomycin or neomycin were either inactive (streptamine, strepturea, methylneobiosaminide) or inhibitory (neomycin B and C, kanamycin, streptidine) to all the classes of streptomycin-dependent mutants. Streptomycin and catenulin, when present alone in concentrations of 5 to 100 μg per ml, gave good growth of the second and third classes of dependent mutants. A mixture of the two antibiotics, however, in any proportion so long as the concentrations surpassed 2 μg per ml, prevented growth of the organisms. The dependent cells grown in the presence of one antibiotic behaved as sensitive cells in respect to the other, indicating diverse mechanisms of growth-promoting action for streptomycin and catenulin. Neamine exhibited similar nutritional incompatibility with various streptomycins.

Hashimoto (1955) obtained a partially streptomycin-dependent strain from the parent strain of pneumococcus I by successive cultivation in media containing increasing amounts of streptomycin. This strain gave the same amount of bacterial growth on media containing no streptomycin and on media with 1 mg per ml of streptomycin, but the growth was faster in the latter. Crude deoxynucleic acid prepared from the partially streptomycin-dependent strain could bring about transformation of the streptomycin-indifferent strain in similar ratio. The deoxynucleic acid was believed to have a factor that controlled the dependence on 50 to 100 μg of streptomycin and that was imparted to the streptomycin-sensitive strains.

Streptomycin-dependent cultures also show back mutations. Yegian and Budd (1951) obtained from such strains of *Mycobacterium ranae* either parent sensitive cultures or streptomycin-resistant variants, with no discernible change in colony morphology.

Thind (1958) reported that extracts of certain streptomycin-sensitive cultures of *E. coli* produce a substance which can replace streptomycin in supporting the growth of streptomycin-dependent organisms.

Chapter 10

Development of Resistance

Natural and Acquired Resistance

The problem of increased bacterial resistance to chemotherapeutic agents has been the focus of special attention in recent years, with increasing utilization of antibiotics for the control of a variety of bacterial infections. The first two important antibiotics, penicillin and streptomycin, have proved to be of particular interest. They are similar in some respects and different in others. Both act primarily upon bacteria, have little effect upon intracellular parasites (rickettsiae), and act not at all or to only a very limited extent upon fungi. They differ, however, in their respective antibacterial spectra. Penicillin is active largely against cocci, gram-positive aerobes and anaerobes, and spirochetes, but has only a limited effect upon gram-negative rods and acid-fast bacteria unless used at high concentrations. Streptomycin, on the other hand, is effective against both gram-negative and gram-positive bacteria, including the acid-fast organisms, and is relatively less active against the cocci and spirochetes than is penicillin. Bacteria develop resistance to streptomycin much more rapidly than to penicillin and may lose that resistance much more slowly (Finland, 1956).

Different strains of the same species exhibit considerable variation in their sensitivity to a given antibiotic. Staphylococci show wide ranges of sensitivity to penicillin. The sensitivity of *Mycobacterium tuberculosis* to streptomycin ranges from 0.1 to 12.5 μg per ml, or 1 to 125. This natural variation in sensitivity of a given organism is of great practical importance from a chemotherapeutic point of view, since it influences the selection of the particular antibiotic for the treatment of a given infection, and the concentrations to be used.

In addition to the natural variation in sensitivity, a population of organisms originally sensitive becomes gradually more resistant or "fast" to a given antibiotic on continued contact with it, either in the test tube or in the body of the host. This phenomenon is not new in either bacteriology or chemotherapy. It has long been observed, for example, that upon repeated administration of a drug, the infecting organism becomes less susceptible to it. This decrease in sensitivity has been assumed to be of two kinds: (1) a reduction of the sensitive strains with a selection of the naturally resistant forms; (2) a change of the sensitive strains into resistant ones.

Ehrlich and others reported, for example, that the resistance of trypanosomes to atoxyl and to dyes could be raised by gradually increasing the doses of the drug. A microbial strain resistant to one type of compound could still be sensitive to other agents. The same organism could be made to develop resistance against several substances, by a series of treatments or adaptations. The ability of various bacteria to become resistant to sulfa drugs has also been well demonstrated. When the bacteria are removed from contact with the drug they become sensitive again; the rate of loss of resistance

varies with the organism, some losing their resistance rapidly and others only very slowly. Certain strains of bacteria may not become resistant to the drug at all. An organism made resistant to one sulfa compound was found to become resistant to others.

With the introduction of antibiotics for chemotherapeutic purposes, it was soon evident that the problem of bacterial resistance would eventually become of paramount importance. It is sufficient to list here a few of the recent contributions to this highly important phase of chemotherapy. Further information is given in the work of Ramsey and Padron (1954), Katsunuma and Nakasato (1954), Knight and Collins (1955), Jones *et al.* (1956), Finland (1958), Chernomordik and Kobeleva (1959), and numerous others.

The Oxford group of investigators (Chain *et al.*, 1940), in their first report on the use of penicillin for disease control, noted a marked increase in resistance of *Staphylococcus aureus* to penicillin upon continued use. This observation was soon confirmed by many other investigators and was found to hold true also for certain other organisms naturally susceptible to penicillin. The occurrence of the natural variation in resistance of bacteria to penicillin was also soon recognized. Bacteria acquire resistance to penicillin when cultivated in a medium containing gradually increasing concentrations of the drug, provided these are kept below the level inhibiting bacterial growth. The tolerance of a strain of gonococcus was increased 350 times and of meningococcus 136 times the concentration originally permitting growth. The increase in resistance of meningococci to penicillin could also be brought about by passage of the culture through penicillin-treated mice. This increase in resistance was not accompanied by the production of the enzyme penicillinase, which has the capacity of destroying penicillin.

Considerable variation has been reported for the development of resistance by bacteria to streptomycin. Of particular importance in this connection is the increased resistance of *M. tuberculosis* isolated from a host that has been treated with considerable quantities of this antibiotic. Variation in sensitivity of *E. coli* to streptomycin was found to range from 0.3 to 3.0 μg per ml, with an average of 1 μg per ml for nine strains. In the case of seven strains of *Proteus vulgaris*, the variation was from 0.3 to 2.5 μg per ml. Similar variations were obtained for other bacteria. A strain of *Pr. vulgaris* made resistant to streptomycin showed only a slight increase in resistance to streptothricin, a closely related antibiotic.

Bacteria develop resistance to streptomycin very rapidly. Among bacteria isolated from the urinary tract, it was found that only three to seven transfers were required to make strains of *Pseudomonas* resistant to 1000 μg of streptomycin per ml, and between four and seven transfers for *Streptococcus faecalis*. Two strains of *E. coli* required 7 to 12 transfers. *A. aerogenes* and colon-aerogenes intermediate strains required 2 to 17 transfers. *Proteus* strains were sensitive to 3.1 to 6.2 μg of streptomycin per ml, and from 7 to 11 transfers were required to make them resistant to 1000 μg per ml. When *Proteus* was grown in urine, 12 to 24 transfers were required to make the bacteria resistant to 1000 μg of streptomycin per ml of urine. *Proteus* splits urea, thus increasing the alkalinity of the medium to more than pH 8, which increases the activity of streptomycin.

Streptomycin-resistant strains showed no change in susceptibility to either penicillin or to sulfonamides. This led to the suggestion that a combination of streptomycin with one of these drugs might prove effective. It was possible to increase the resistance of gram-negative bacteria to more than 50,000 μg per ml of streptomycin by passage through increasing concentrations of this antibiotic. In

most instances this resistance was increased gradually. However, in some cases, there was a sudden increase in resistance of isolated colonies from relatively low values to more than 50,000 μg per ml. When a culture of organisms was made resistant by exposure to streptomycin in broth, some of the cells showed marked pleomorphism and in some instances underwent changes in biochemical reactions. However, when resistant organisms were obtained from a patient during or after treatment, no morphological or cultural differences were observed, as compared with the sensitive strains isolated from the same patient.

The development of resistance of fungi to polyenic antifungal antibiotics has been studied by Stout and Pagano (1955) and by Pledger (1957). These authors showed that by repeated transfer in the presence of polyenes the resistance of naturally sensitive fungi was only slightly increased. Pledger, for instance, reported that after 15 to 38 transfers in the presence of candicidin, candidin, filipin, and nystatin, the resistance of strains of *C. albicans* and *Sacch. cerevisiae* increased only 1.5- to 8.3-fold.

Mechanism of Development of Resistance

Various explanations have been suggested for the development of resistance. These can be summarized as follows: (1) Induced resistance is due to the killing of sensitive cells in a given bacterial population, which permits the more resistant cells to grow selectively. (2) The phenomenon of resistance is due to the acquisition of new enzyme systems or to new metabolic activities which permit the organism to survive in spite of the presence of the particular inhibiting agent. (3) Certain treatments tend to reverse the effect of resistance or to prevent its occurrence altogether.

Davies, Hinshelwood, and Pryce (1944) expressed the above concepts of the development of adaptation of an organism to an antibacterial agent as follows: (1) Adaptation occurs by natural selection from an initially heterogeneous population. (2) Adaptation occurs by the modification of the individual cells, as a result of the establishment in the cells of a mechanism alternative to that normally in use, or to a quantitative modification of the existing mechanism. (3) Adaptation is a change in some center of organization of the cell. When variations or adaptive changes occur, there is an actual modification of the character of the individual cells, although selection may be superimposed on this when modified and unmodified cells exist together. Postgate and Hinshelwood (1946) found that most consideration should be given to the hypothesis that qualitative and quantitative changes occur in the cell enzymes in response to the changed medium.

The development of resistance among bacteria sensitive to particular antibiotics, on contact with such antibiotics, is sometimes highly specific; those bacteria that become resistant to one antibiotic may still remain sensitive to another. Staphylococci which develop resistance to penicillin are not affected in their sensitivity to streptomycin or to various other antibiotics.

When an organism develops resistance to one antibiotic and yet remains sensitive to another, the modes of action of these two compounds are assumed to be different. According to Chain and Florey (1944), the formation by a given organism of strains resistant to different antibiotics serves to emphasize "the great variety of ways in which the organization of the cell is open to attack by chemical substances."

Bryson and Demerec (1955) analyzed the phenomenon of the development of resistance to drugs; this was considered to be an important aspect of the continuing process of microbial evolution. The use of antibiotics resulted in the selection of new types

of organisms by the principle of survival of the fittest. These investigators considered the changes leading to the development of resistance not as primarily drug-induced but rather as a result of spontaneously occurring mutations, leading to modified biochemical processes in the bacterial cell and thereby yielding resistant strains. Two types of resistance were recognized: natural and acquired. The first occurs in a natural population, among species or strains that have had no previous contact with a given antibiotic (Table 36). The second takes place in a bacterial population that has been in contact with an antibiotic; resistant cells emerge from an originally sensitive population.

The emergence of antibiotic resistance was regarded as essentially a phenomenon of adaptation, of which two categories were recognized: (1) genetic adaptation, in which resistant mutants overgrow the population under the selective effect of the antibiotic; (2) physiological, or phenotypic, adaptation, in which cytoplasmic alterations (adaptive enzyme formation) are induced by the antibiotic, so as to render some of the cells more resistant without affecting the genetic apparatus (see also Garrod, 1950).

Some organisms, including resistant species and naturally resistant strains, were believed to possess cytochemical systems that are not vulnerable to specific antibiotics. Degrees of resistance and sensitivity were considered as relative. Two resistance patterns were recognized: (1) the penicillin or obligatory multistep pattern; (2) the streptomycin or facultative one-step pattern. The nature of the pattern permits prediction of the probability that resistance will develop rapidly. Mutations to antibiotic resistance were believed to include a wide variety of types. Gain or loss of resistance, once mutations to resistance have occurred, depends primarily on selection, the presence or absence of antibiotics playing a major part (Bryson and Demerec, 1955).

TABLE 36

Approximate concentration required to produce detectable inhibition of cells in streaks of representative bacterial species on gradient plates (Bryson and Demerec, 1955)

Escherichia coli		*Staph. aureus*		*Mycobacterium ranae*		*Bacillus megaterium*	
Drug	μg/ml	Drug	μg/ml	Drug	μg/ml	Drug	μg/ml
Bacitracin	700	Viomycin	80	Chloramphenicol	20	Viomycin	10
Viomycin	50	Licheniformin B	50	Streptothricin	15	Streptothricin	8
Netropsin	8	Bacitracin	20	Thiolutin	15	Mycomycetin	3
Catenulin	6	Netropsin	10	Isoniazid	5	Tetracycline	1.5
Penicillin	5	Mycomycetin	10	Netropsin	4	Carbomycin	0.8
Streptothricin	4	Streptothricin	4	Viomycin	2	Oxytetracycline	0.8
Neomycin	2	Chloramphenicol	4	Catenulin	2	Streptomycin	0.7
Streptomycin	2	Streptomycin	3	Streptomycin	0.6	Erythromycin	0.2
Chlortetracycline	1	Thiolutin	3	Neomycin	0.5	Neomycin	0.04
Oxytetracycline	1	Catenulin	1	Oxytetracycline	0.4		
Chloramphenicol	1	Neomycin	0.8	Mycomycetin	0.2		
		Oxytetracycline	0.5	Chlortetracycline	0.05		
		Chlortetracycline	0.3				
		Penicillin	0.02				

A growing culture may acquire resistance to an antibiotic by selection of cells initially less sensitive than the average, or by a sudden modification of the properties of individual cells, namely, by mutation. According to Demerec (1950), resistance by *Staph. aureus* to streptomycin originates by gene mutation. Differences in the development of resistance to penicillin and to streptomycin were observed by plating cultures of various organisms in the presence of increasing concentrations of either antibiotic. The penicillin-resistant strains produced in the first step were fairly uniform in their degree of resistance; on the other hand, streptomycin-resistant strains showed great variability, some highly resistant forms being obtained in one step. It was suggested that penicillin mutations may be a result of a number of genes, whereas in streptomycin mutations some genes are more effective than others.

In the development of our knowledge of resistance of microorganisms to antibiotics, several phenomena have been recognized:

1. Microorganisms develop resistance to antibiotics by different mechanisms; such development of resistance takes place in different ways, as in the case of penicillin *versus* streptomycin type of resistance.

2. Some antibiotics show cross-resistance, as in the case of tetracyclines and chloramphenicol, and among members of the neomycin group; others do not, as in the case of penicillin and streptomycin, where an organism developing resistance to one antibiotic remains sensitive to another.

3. After continued use of a certain antibiotic, there is a gradual development of resistance among the bacteria that formerly were highly sensitive to this antibiotic. The occurrence of staphylococci resistant to penicillin has reached more than 60 per cent in some hospitals. This is also true of other bacteria and other antibiotics, as shown in Table 37.

TABLE 37

Propensity of microorganisms to develop antibiotic-resistant strains (Dowling *et al.*, 1955)

Group	Microorganism	Rapidity of response to proper antibiotic treatment	Frequency of appearance of resistant strains in patients during treatment
1	Pneumococci Meningococci β-Hemolytic streptococci (excluding group D streptococci) Gonococci *Shigella* *Hemophilus influenzae*	Rapid	Seldom, if ever
2	*Staphylococcus* *Str. viridans* Enterococci *Proteus* *Pseudomonas* group Coliform bacteria *Mycobacterium tuberculosis*	Occasionally rapid; usually slow or incomplete	Often
3	*Brucella* *Salmonella typhosa* Rickettsiae	Rapid; frequently followed by relapse	Seldom, if ever

4. By combining two antibiotics, such as streptomycin with penicillin, or an antibiotic and a chemical agent, such as streptomycin with *p*-aminosalicylic acid or isoniazid, resistance to any one agent can be delayed if not prevented.

5. Little is known concerning the mechanism of development of resistance. The fact that treatment of sensitive cells with deoxyribonucleic acid isolated from resistant cells renders the sensitive cells resistant, and similar observations, may suggest proper approaches in overcoming the development of resistance.

The background of the problem of development of resistance of microorganisms to drugs, comprising both natural and acquired resistance, has been further analyzed in detail by Abraham (1953), Schnitzer and Grunberg (1957), DiMarco (1958), and others. Abraham (1959) considered the ability of bacteria to acquire resistance to antibiotics as one of the many examples of the adaptability of microorganisms, involving the basic problems of protein and nucleic acid synthesis. The procedure of Lederberg and Lederberg (1952), whereby samples of a mass of bacteria are first transferred from the surface of a nutrient agar plate to a velvet pad and then printed on other plates to give replicas of the first, suggested that bacteria highly resistant to streptomycin can be formed without any contact with the drug. The fact that successive prints from a normal plate of *E. coli* to plates containing streptomycin show a lack of multiplication of most of the organisms, but give growth of a small number of colonies of resistant cells suggests that resistant cells, formed by rare mutations, already exist on the normal plate and are later selected in the presence of the drug. Abraham added, however, that this does not prove that random mutation represents the only mechanism by which resistance to antibiotics develops.

Lederberg in 1959, said:

> In some favorable instances the spontaneous origin of drug-resistant mutants can be verified unambiguously by contriving to isolate them without their ever being exposed to the drug. One method entails indirect selection. To illustrate its application, consider a culture of *Escherichia coli* containing 10^9 bacteria per milliliter. By plating samples on agar containing streptomycin, we infer that one per million bacteria or 10^3 per milliliter produce resistant clones. But to count these clones they were selected in the presence of streptomycin which hypothetically might have induced the resistance. We may, however, dilute the original bacteria in plain broth to give samples containing 10^5 per milliliter. Since 10^{-6} of the bacteria are resistant, each sample has a mathematical expectation of 0.1 of including a resistant bacterium. The individual bacteria being indivisible by dilution, nine samples in ten will include no resistants; the tenth will have one, but now augmented to 10^{-5}. Which one this is can be readily determined by retrospective assay on the incubated samples. The procedure can be reiterated to enrich for the resistant organisms until they are obtained in pure culture. The same result is reached more conveniently if we spread the original culture out on a nutrient agar plate rather than distribute samples into separate test tubes. Replica plating, transposing a pattern of surface growth from plate to plate with a sheet of velvet, takes the place of assaying inocula distributed in tubes. Dilution sampling and replica plating are then alternative methods of indirect selection whereby the test line is spared direct contact with the drug.

According to Leidy *et al.* (1956), streptomycin resistance of a high degree can be induced in sensitive populations of *Hemophilus influenzae* and *H. parainfluenzae* by deoxyribonucleic acids (DNA) derived from streptomycin-resistant cells of at least one heterologous species of *Hemophilus*. Comparison of the activity of heterologous and homologous DNA showed differences within species and degrees of differences among species not brought out by other available methods. According to these results, *H. influenzae* is more closely related to *H. parainfluenzae* than to *H. suis*, the relationship between the last two being remote. The low proportion of cells in *H. influenzae*

populations which are made streptomycin-resistant by DNA derived from streptomycin-resistant *H. parainfluenzae* and *vice versa* has been increased 4- to 15-fold by the replication of the heterologous species streptomycin-resistant DNA in the heterologous species. An alteration of the heterologous DNA by the host was suggested.

Lightbown (1957) made a study of the development of resistance to streptomycin by *Ps. pyocyaneus*. Resistance was found not to be enzymatic, but to be due to the production of alkyl-substituted quinoline-N-oxides, which are potent inhibitors of bacterial cytochrome electron transport at concentrations that give increased resistance to streptomycin for organisms such as *B. subtilis* and *Staph. aureus*. Resistance to streptomycin was believed to depend on the development and choice of alternative pathways of metabolism; the effects of the quinoline-N-oxides may be due to the inhibition of the Pasteur effect, allowing more active glycolysis to occur aerobically. Obligate aerobes were believed to possess an alternative to the cytochrome terminal pathway. This may be, in part, a flavoprotein oxidase-peroxidase path. Certain elements, notably Mg, accelerate the development of resistance to streptomycin; others, like Co, have an inhibitory effect (Chernomordik and Kobeleva, 1959).

Pollak (1956) studied antibiotic resistance of *M. kansasii*, an atypical acid-fast organism previously known as the "yellow bacillus." It was as susceptible to streptomycin as *M. tuberculosis*, but about 5 to 10 times as resistant to *p*-aminosalicylic acid and isoniazid.

Sebek (1958) investigated growth-inhibitory effects of neomycin B, neomycin C, and neamine on 24 bacterial species (Table 33). Particularly striking differences were obtained in *Corynebacterium* sp. and *Sarcina lutea*. The growth of the first was inhibited by about 250 μg per ml of neamine or neomycin C but by only 0.4 to 1.6 μg per ml of neomycin B. When neomycin C and neamine were added in varying ratios and in different combinations to neomycin B, the inhibitory concentrations of neomycin B remained virtually unchanged. The organism readily acquired resistance and cross-resistance to a high degree to neamine and neomycin C but only slightly to neomycin B. It was suggested that the diaminohexose portion of neomycin B is responsible for the specific growth-inhibitory effect of this antibiotic on these two bacteria. Cross-resistance between streptomycin and neomycin has been studied by Sidi *et al.* (1958).

Several cross-resistant groups are now recognized among the antibiotics of actinomycetes: (1) streptomycin, streptothricin, viomycin, and neomycin, as well as kanamycin, catenulin, and paromomycin; (2) the tetracyclines, chloramphenicol, and possibly penicillin; (3) erythromycin, carbomycin, celesticetin, oleandomycin, and spiramycin.

Kunin *et al.* (1958) observed that kanamycin, paromomycin, and neomycin had essentially the same activity against strains of *Staph. aureus* and of various *Enterobacteriaceae*. Bacteria made resistant to any one of these three antibiotics by subcultures on that antibiotic also exhibited complete cross-resistance to the other two. Freshly isolated cultures did not show significant cross-resistance between streptomycin and these three antibiotics; however, strains made resistant to any one of the three also showed increases in resistance to streptomycin; strains made resistant to the latter exhibited only minor increases in resistance to the others. Cultures of staphylococci comprising both parent and resistant (to all four antibiotics) variants were of the same phage type; resistant variants of *Kl. pneumoniae* and *E. coli*, however, retained their serological specificity. During oral treatment with paromomycin or kanamycin, fecal organisms were resistant to the antibiotic

administered and also showed moderate to marked resistance to the other one and to neomycin.

Finland (1958) also found cross-resistance between kanamycin, paromomycin, and neomycin, but little or none between these and streptomycin. The fact that they all show 8th cranial nerve toxicity and renal toxicity was believed to indicate further the relationship between chemical structure and pharmacological properties.

Welsch (1957), who examined the problems of resistance facing the clinician, emphasized that the antibiotics should be used with the utmost care. He concluded that bacterial resistance takes place at three different biological levels—the species, the strain, and the individual cell—with different clinical implications in each instance:

1. Variations of resistance at the level of the species account for the occurrence of superinfections as the result of a selective destruction of some organisms of the normal flora. The clinician must thus make a first approximation of the antibiotic spectrum of the causative organism for emergency therapeutics.

2. Differences of resistance between natural strains within a given species are very marked in staphylococci. The heterogeneity of the species accounts for the results obtained from the widespread use of an antibiotic, in the selection of resistant strains, and the occurrence of resistant cross-infections in a treated individual.

3. The occurrence of spontaneous mutants accounts for variation of resistance among individuals of a pure bacterial population. It was suggested that environmental factors might influence the phenotypic expression of individual resistant mutants derived from the same clone. This hypothesis is based on the fact that the degree of resistance observed is not necessarily quantitatively related to the concentration of the drug to which the bacteria were submitted. Variation of resistance at the level of the individual cell accounts for the progressive ineffectiveness of chemotherapy sometimes observed during treatment. The practical importance of this type of resistance was said to be limited largely to streptomycin therapy and to treatment of tuberculosis. The use of an association of chemotherapeutic agents is known to minimize the emergence of resistant organisms.

Welsch further emphasized that organisms surviving the bactericidal action of an antibiotic need not necessarily be resistant to the drug, as in the case of penicillin and streptomycin. Further, exposure of bacteria to an association of antibiotics does not necessarily prevent the emergence of resistant individuals.

Bacterial cultures with resistance acquired *in vitro* show certain altered biological characteristics, such as partial or complete dependence on the antibiotic, decreased viability, retarded growth rate, slower metabolic reactions, decreased or altered nutritional requirements, certain pleomorphic changes, changed cultural properties, changed staining reactions, and frequently a reduction in virulence.

Bacterial cells made resistant to antibiotics grow at a lower rate and produce less growth in simple and complex media than do the original sensitive strains. When the resistant strains are grown in the presence of the sensitive parent strains but in the absence of the corresponding antibiotic, the former are suppressed, especially in simple media. This was also recently established by Blackwell and McVeigh (1960) in their study of the effect of dihydrostreptomycin upon *E. coli*.

Sevag and Rosanoff (1952) demonstrated that the synthesis of phenylalanine and aspartic acid by sensitive cells of *Staph. aureus* is blocked by streptomycin. These amino acids are synthesized by both sensitive and resistant cells in the absence of streptomycin.

Practical Aspects

The recognition of potentialities of increasing resistance of bacteria to a given antibiotic, thus resulting in a decrease in the therapeutic value of the antibiotic, led to rather alarming generalizations. This was brought out by Molitor (1946), who said, "Whether the high incidence of drug-fastness is due to the liberal and indiscriminate use of these new, practically non-toxic agents, or whether these drugs are particularly likely to produce resistant strains, is not known. Regardless of the cause, however, there is the prospect that in the relatively near future penicillin and streptomycin may to a considerable degree lose their usefulness in the therapy of some of the most prevalent infections unless some means can be devised to restore the original susceptibility of either host or pathogenic agent."

In an effort to overcome the development of drug resistance, it has been suggested that the selected dose of the antibiotic be large enough to eliminate the pathogenic organisms from the body rapidly. Penicillin and streptomycin, because of their low toxicity, make possible the administration of doses greater than those required to stop bacterial growth. It is necessary to determine the resistance of the pathogen prior to the treatment. High initial concentrations of the antibiotic insure the maintenance of high blood concentrations. It has been emphasized that use of the antibiotic in such preparations as salves, lozenges, chewing gum, and sprays is likely to produce adequate concentrations only at the site of application and would tend to create a hazard unless special forms can be developed which will assure a completely adequate drug concentration in blood and body tissue.

The possibility of developing new antibiotics offers further promise of overcoming the resistance of an organism to a given antibiotic. This makes possible the combined use of two substances, which would tend to repress the few resistant cells. It has been shown, for example, that use of penicillin and bacteriophages in combination produces a synergistic effect. Malignant infections due to staphylococci, colon bacilli, and certain streptococci showed good response to such a combination.

Combinations of Antibiotics

Among the procedures recommended for overcoming the problem of bacterial resistance, combined therapy of two or more drugs has been given particular consideration. Such combined activity may be synergistic, additive, indifferent, or antagonistic, depending upon the nature of the antibiotics and upon the bacterial species or strain.

Cavalli-Sforza and Lederberg (1953) analyzed in detail the phenomenon of combination of antibiotics. They considered true synergism (the joint effect being greater than expected on simple addition of effects), simple addition, indifferent effect, and antagonism. Physiological synergism was believed to be negatively correlated with genetic synergism (Klein and Schorr, 1953). Additive interaction could be expected between chloramphenicol and tetracycline, which show cross-resistance and therefore genetic antagonism. Tetracycline and penicillin show genetic synergism and physiological antagonism. This was believed to be due to the fact that penicillin has a bactericidal effect only on growing cells, and a bacteriostatic agent would therefore greatly reduce its action. The association of chloramphenicol and sulfa drugs and the association of isoniazid and streptomycin in the therapy of tuberculosis were considered as involving both genetic and physiological synergism.

Jawetz and Gunnison (1953) made a detailed study of the synergistic or additive properties of such combinations. Unfortunately, there was frequently a lack of correlation between the activities of the combina-

tions *in vitro* and *in vivo*. Jawetz (1958) emphasized that the basic mechanisms underlying combined antibiotic action are still unknown. He agreed, however, that certain combinations may give good results in clinical practice. This conclusion is based upon laboratory data and clinical judgment. He outlined the rational use of two antimicrobial drugs, instead of one, as follows:

1. In mixed infections it is possible that two drugs, each acting on a separate portion of the complex microbial flora, might be more effective than one drug. This applies occasionally to infections of skin, wounds, or body cavities, particularly when nonabsorbable drugs of limited antibacterial spectrum are used topically (*e.g.*, polymyxin, bacitracin, or neomycin).

2. Toxic side effects may sometimes be reduced by employing simultaneously two drugs which have a similar antibacterial action but distinct toxic effects. A combination of such drugs could obtain a given antibacterial effect together with a lower toxicity than would be feasible with either of the components of the mixture used alone. Streptomycin-dihydrostreptomycin mixtures may serve as an example.

3. In some clinical situations the rapid emergence of bacteria resistant to one drug may impair the chances for cure. The addition of a second drug sometimes delays the emergence of resistance. This effect has been demonstrated unequivocally in tuberculosis. In some other chronic infections the evidence for its occurrence is questionable. In serious systemic staphylococcal infections, streptomycin, erythromycin, novobiocin, or related drugs should not be used singly, as a rule, because resistance to each is likely to emerge rapidly.

4. In certain desperately ill patients with suspected infection of unknown etiology it may be desirable to administer several antimicrobial drugs after all steps have been taken to establish an etiologic diagnosis. These drugs are aimed at the organisms most likely to cause the clinical picture encountered and are usually continued only until the discovery of an etiologic agent permits specific therapy. The initial treatment of meningitis in a small child might be an example in this category.

5. In some infections the simultaneous use of two drugs gives an effect not obtainable by either drug alone. Perhaps the best established example is endocarditis due to *S. faecalis*, for which the combined effect of penicillin and streptomycin is essential for cure. The "synergistic" drug effects and the known dynamics of combined drug action are summarized later (Chapter 11).

Chapter 11

Utilization of Antibiotics in Clinical Medicine and Other Applications

Before the advent of modern chemotherapy, the treatment of disease in general and of infectious diseases in particular by means of chemical agents was arbitrary and fragmentary in nature. In most instances, justification for the particular method of treatment was speculative rather than scientific.

The use of plant products offers an interesting illustration. Since ancient times or long before the role of microbes in the causation of infectious diseases was recognized, certain plants have been used for the treatment of various infections. Reference to this is found in the Herbals of the Chinese, in the Ebers papyrus of Egypt (the use of onions), in the Old Testament (Isaiah advised the use of figs for the treatment of a boil of Hezekiah), in the writings of ancient Greece (Theophrastus) and Rome (Dioscorides), as well as in folk medicine in South Africa and in Central and South America. Cinchona bark has been used in the treatment of malaria. Chaulmoogra oil for leprosy and the use of certain alkaloids may be cited as other classic examples of plant products that have found, even up to recent times, extensive application in the treatment of various diseases. Mention may also be made of the use of cepharantine in Japan in the treatment of tuberculosis. Among the other plant products that possess marked antimicrobial substances, the phenolic compounds occupy a prominent place.

We now know that various plants produce chemical substances which possess antibacterial properties. However, it is neither these nor the enzyme-rich plants (such as figs) that have found extensive application in the treatment of infections caused by microbes, but rather the products of microbes themselves, namely, the antibiotics.

At first, to combat infections, came the use of certain preparations, in the form of vaccines, serums, and antitoxins, obtained from the causative microbes. These preparations were used, and many still continue to be used, either as prophylactics or as therapeutics. They range from common cold and smallpox vaccines to Calmette-Guérin bacillus (BCG) and polio vaccines, from diphtheria and tetanus antitoxins to antistaphylococcal serums. Strictly speaking, however, none of these come under the category of chemotherapy. They are to be considered as immunotherapeutic methods rather than as chemotherapeutic procedures. This is true despite the fact that some of these preparations, such as the diphtheria and tetanus antitoxins, have been isolated in a purified chemical state.

The field of modern chemotherapy is based largely upon the discovery of four types of compounds: (1) salvarsan and other arsenicals; (2) sulfanilamide and other sulfa drugs, including the sulfones; (3) other synthetic chemical compounds, notably *p*-aminosalicylic acid and isoniazid; (4) the antibiotics.

It is the last group with which we are concerned here.

It has been said that much greater progress has been made in the treatment of infectious diseases during the last 15 or 20 years than in all previous medical history. The first real progress in combatting infectious diseases was made during the latter part of the last century with the discovery of immune sera and antitoxins. This was followed by the work of Paul Ehrlich and others on the great potentialities of the arsenicals as chemotherapeutic agents, resulting in the discovery of salvarsan in 1910. A quarter of a century later came the sulfonamides, broader in scope and of much wider application. They were soon followed by discovery of the antibiotics, of which penicillin and streptomycin were the first two most striking examples.

The mere listing of the various uses of antibiotics is sufficient to emphasize their broad application.

I. Treatment of infectious diseases.
   A. Infectious diseases of man.
      1. Diseases caused by gram-positive bacteria; penicillin, chloramphenicol, tetracyclines, erythromycin, and various others are used.
      2. Diseases caused by gram-negative bacteria (gastrointestinal infections, tularemia, plague, cholera, and numerous others); streptomycin, neomycin, chloramphenicol, tetracyclines, and certain others are effective.
      3. Various forms of tuberculosis; streptomycin and certain other antibiotics have found particular application.
      4. Diseases caused by rickettsiae and the psittacosis-lymphogranuloma group of organisms; the tetracyclines, chloramphenicol, and erythromycin are used.
      5. Diseases that can be treated effectively by combinations of antibiotics with synthetic compounds.
   B. Numerous animal diseases that afflict poultry, swine, dogs, cats, and other domesticated animals.
   C. Plant diseases caused by bacteria and certain fungi.
      1. Fireblights and other bacterial diseases of trees.
      2. Bacterial diseases of vegetables, such as string beans and peppers.
      3. Bacterial diseases of flowers, such as carnations.
      4. Diseases caused by fungi, such as the blue mold of tobacco.
II. Treatment of diseases, noninfectious in nature, notably neoplasms.
III. Preservation of valuable biological products.
   A. Virus preparations.
      1. Poliovirus.
      2. Poultry vaccines
   B. Human and animal semen.
   C. Foodstuffs, notably fish and poultry.
IV. Animal feeding.

More than 30 antibiotics are now available for the treatment of various infectious diseases caused by bacteria, fungi, protozoa (amoebae, trichomonads, etc.), rickettsiae, the psittacosis-lymphogranuloma group, and certain worms. Some are more effective than others. Some are best used orally, others by injection, still others by surface application. The development of resistance against one necessitates the use of others. Undesirable reactions caused by a particular antibiotic through one method of administration, as in the case of neomycin used parenterally, suggest other methods of administration, as oral or topical for neomycin.

Animal experiments cannot always be considered as true representations of the chemotherapeutic potentialities of a given

antibiotic in man. This has been emphasized, for example, for cycloserine (Mulinos, 1955).

A partial list of antituberculosis agents produced by microorganisms would indicate among the bacterial products nisin, licheniformin, and viscosin. These were soon discarded, however, in favor of others. Among the fungal products, only clitocibin appeared at first to offer promise, but it was discarded as well. The actinomycetes eventually yielded the most valuable products. The most important of these is streptomycin, followed by viomycin, cycloserine, neomycin, and kanamycin (Scowen, 1960).

The development of resistance among the staphylococci to penicillin, especially under hospital conditions, has led to a concentration of attention on this growing problem in the use of antibiotics (Lowbury, 1960; Pollock, 1960). Some pessimists have gone so far as to predict that within 10 years the antibiotics will no longer be clinically useful because all bacteria will have developed resistance to these drugs. Such predictions are the height of absurdity and ignorance. Even the "hospital staphylococci" would not have developed resistance so rapidly if the principles of cleanliness had been adhered to and if the antibiotics had not been expected to take the place of cleanliness; if self-healing by many patients had been strictly controlled; and if excess use of antibiotics had been avoided. Perhaps the warning came in good time. Fortunately, other antibiotics have come to supplement or take the place of those to which bacteria have developed resistance.

Treatment of Infectious Diseases

The introduction of antibiotics in the treatment of infectious diseases completely revolutionized medical science and medical practice. Diseases like cholera, plague, dysentery, typhoid, and typhus fever, which formerly at frequent intervals decimated the human race, and the numerous diseases of childhood have now been brought under practical control. Diseases that only two decades ago were believed to be beyond the control of man are now being successfully treated. Many of them have been almost completely eradicated. Tuberculosis, formerly known as consumption or as the Great White Plague, has receded in a rather short period of time from first to tenth place as a killer of mankind. First to be found effective in its treatment was streptomycin. This was later supplemented by certain synthetic compounds (*p*-aminosalicylic acid and isoniazid) and other antibiotics (viomycin, cycloserine) to increase the effectiveness of this agent (Crofton, 1960). As a result of the tremendous developments due largely to antibiotics, the attitude of the average man to disease, especially to infectious disease, has changed in one generation from fear to understanding.

In deciding upon a particular antibiotic for the treatment of a given infection, the close collaboration of the clinic and laboratory is most essential (Gould, 1960). The use of antibiotics in chemotherapy is based upon the assumption that the drugs act upon the infectious agents without injuring the host and without affecting the natural defenses against the infectious agent. It is further based upon the close correlation of the effect of antibiotics upon sensitive organisms *in vitro* as opposed to their effect *in vivo*. Sensitivity tests must therefore be very accurate. A recent analysis of the problems involved was made by Ericsson and Svartz-Malmberg (1959).

Two important factors must be considered: (1) variation in sensitivity of individual strains of the same organism, and (2) development of resistance. On the basis of the clinical response to antibiotic therapy and rapidity of development of resistance, Dowling *et al.* (1955) classified disease-producing organisms in three groups, as shown in Table 37.

Group 1 comprises organisms that usually respond promptly to antibiotics. Resistant

forms are produced with difficulty, and even more rarely in clinical use.

Group 2 includes organisms that often respond only slowly to therapy with antibiotics. In some cases, such as pulmonary tuberculosis or staphylococcal osteomyelitis, patients may improve and yet retain the organisms and infection for years, never becoming entirely well. Resistant forms are also often encountered.

Group 3 includes organisms causing brucellosis, typhoid, and some rickettsial infections. The organism isolated during recurrence remains sensitive to the antibiotic originally used, and the infection will respond to another course of therapy.

Antibiotic resistance is particularly serious in the case of staphylococci, tubercle bacilli, and certain gram-negative forms, such as *Proteus*, *Pseudomonas*, and the coliform organisms. Staphylococcal infections due to resistant strains are said to constitute the most serious clinical problem of antibiotic resistance. The solution of the problem of the emergence of antibiotic-resistant staphylococci has received much attention (Dunlop, 1960). Combination therapy with two antibiotics has been recommended, provided the organism is sensitive to each antibiotic alone. Unfortunately, there is no evidence that the incidence of resistant strains is always reduced by combined therapy. Lepper *et al.* (1956) used novobiocin plus spiramycin in a routine manner for 6 months. A gradual increase in the incidence of staphylococcus resistance to each antibiotic occurred despite this combination therapy (see also Kass, 1955, and Velu, 1958). The preventive use of antibiotics must also be mentioned (Bywaters, 1960; Taylor, 1960).

Animal Diseases

On a par with the revolution that took place in the treatment of infectious diseases in man as a result of the introduction of antibiotics, one must consider the remarkable role played by these agents in the treatment of infectious diseases in animals. Brucellosis and mastitis in cattle and numerous infectious diseases of dogs, cats, poultry, and other domesticated animals can now be controlled by antibiotics.

Animal Nutrition

In addition to their use for the control of infectious diseases, antibiotics have also been employed extensively in the nutrition of nonherbivorous animals, such as poultry and swine. This effect is particularly marked in animals deprived of β-carotene and vitamin A (Guerrant, 1960). For further details, see Jukes (1955) and Goldberg (1959).

Among the uses of antibiotics that involve both animal feeding and disease control, one might mention the control of silkworm diseases by the soaking of mulberry leaves in antibiotic solutions. The use of streptomycin and tetracyclines resulted not only in the almost complete suppression of bacterial infections in silkworms, but also resulted in a higher quality silk (Afrikian, 1960).

Food Preservation

In recent years, certain antibiotics, notably the tetracyclines, which are readily destroyed on boiling, have been used extensively in the preservation of fish and poultry products (Carey, 1958).

Laboratory Uses

Numerous laboratory processes have benefited greatly from the utilization of antibiotics. One might mention their use in the preparation of selective culture media for the isolation of (1) gram-negative bacteria, (2) anaerobic bacteria, (3) fungi, (4) protozoa, and (5) viruses. They are used in genetics for the isolation of special nongrowing variants which depend upon specific growth factors. They are used for preservation of cattle semen and virus preparations. Finally, their use in tissue cultures and in the produc-

tion of virus vaccines has made possible certain special forms of prophylactic therapy.

Problems Arising from Use of Antibiotics

The tremendous benefits of antibiotics to the human race were soon fully recognized. They were lauded as the "miracle drugs." The medical profession was ready to replace the concept of "chemotherapy" with that of "antibiotic therapy." On the other hand, many persons went to the other extreme. If no rapid cures for cancer or for the various viral diseases were forthcoming, they tended to blame the antibiotics. When of the hundreds of thousands of cultures tested and the numerous antibiotics isolated, only 20 or 30 found a place in practical therapy, many persons felt cheated and asked for more. Further, the development of bacterial resistance to some antibiotics and the undesirable reactions occasionally produced in certain patients were given undue publicity, with the resulting designation of the antibiotics as "toxins" and "poisons" (Gale, 1960).

Gradually, however, the undesirable characteristics of the antibiotics are being eliminated through a better understanding of their use. Combinations of two antibiotics, such as penicillin and streptomycin, or of two forms of the same antibiotic, such as streptomycin and dihydrostreptomycin, or of an antibiotic with a synthetic chemical agent, as streptomycin with *p*-aminosalicylic acid or with isoniazid in the treatment of tuberculosis, have tended to increase the effectiveness of antibiotics and decrease their limitations (Lacey, 1960). New antibiotics are constantly being introduced to supplement those now in use. Both the physician and the patient have come to recognize that every new discovery carries with it an obligation and that no sure cure can be expected, even from the most nearly ideal drugs, unless their mode of action, their specific role in therapy, and especially their limitations are thoroughly understood.

Treatment of Plant Diseases

The antagonistic effects of microbial saprophytes upon plant pathogens were recognized long before the advent of antibiotic therapy. Various bacteria and fungi were utilized for the purpose of controlling plant diseases. The results were not always convincing, however, and could never be properly duplicated. On the other hand, some of the antibiotics, such as streptomycin, have proved to be markedly effective in the treatment of various bacterial diseases of plants; and actidione, antimycin, and streptomycin have found a place in the treatment of certain fungus diseases. A detailed review of this subject was recently published by P. Müller (1959).

Important Therapeutic Applications

A list of the antibiotics of actinomycetes that have found *important* therapeutic applications is given here in order of their discovery:

Actinomycins C and D, used in the treatment of certain forms of cancer, such as rhabdosarcoma and Wilms' tumor.

Streptomycin and dihydrostreptomycin, used in the treatment of tuberculosis and of various infections caused by gram-negative and gram-positive bacteria; also of certain plant diseases caused by bacteria and a few fungi.

Chlortetracycline, oxytetracycline, and tetracycline, used in the treatment of diseases caused by various gram-positive and gram-negative bacteria, rickettsiae, and the psittacosis-lymphogranuloma group of organisms.

Chloramphenicol for the treatment of diseases caused by gram-positive and gram-negative bacteria, rickettsiae, and the psittacosis-lymphogranuloma group of organisms.

Staphylococcal infections and typhoid are very susceptible.

Viomycin, which has found a limited use in the treatment of forms of tuberculosis.

Erythromycin, carbomycin, spiramycin, and oleandomycin, active mainly upon gram-positive organisms, particularly penicillin-resistant staphylococci.

Neomycin (framycetin), active upon gram-positive and gram-negative bacteria; largely for topical and oral use, for intestinal sterilization, and for bacterial diarrheas.

Cycloserine for the treatment of resistant forms of tuberculosis and of certain gram-negative urinary tract infections.

Novobiocin, active upon diseases caused by gram-positive organisms, particularly penicillin-resistant staphylococci.

Amphomycin, active upon certain gram-positive organisms; for topical use only.

Ristocetin, active upon gram-positive organisms, especially penicillin-resistant staphylococci; used intravenously only.

Thiostrepton, active upon gram-positive organisms; used orally for intestinal sterilization, usually combined with other drugs.

Hygromycin, used in animal feeds only, for large round worms, nodular worms, whipworms.

Cycloheximide (actidione), used in the treatment of certain plant diseases.

Nystatin, antifungal agent.

Candicidin, trichomycin, used in treatment of diseases caused by fungi.

Amphotericin, active upon fungi causing systemic mycosis.

Vancomycin, active upon gram-positive organisms, particularly penicillin-resistant staphylococci; administered by intravenous injection only.

Kanamycin, active upon gram-positive bacteria, particularly penicillin-resistant staphylococci, and various gram-negative organisms.

A detailed review of the clinical application of most of these antibiotics has been made recently by Florey (1960).

An Outlook

In view of the large number of actinomycete cultures already isolated and tested, the numerous antibiotics and antibiotic preparations obtained, the great variety of chemical compounds now recognized, and their potential biological activities, one might be inclined to think that the limit to our knowledge of antibiotics of actinomycetes may already have been approached. To those of us, however, who have devoted several decades to the study of the actinomycetes, who have searched for them in numerous soils throughout the world, in peat bogs and in composts, in lakes and in the sea, all our present knowledge appears as a mere beginning. Since a single gram of soil may yield, on proper plating on suitable media, a million or more colonies of actinomycetes, representing numerous species with many metabolic potentialities, one can well appreciate the various biochemical mechanisms involved. Many more species are still to be discovered.

Aside from their importance in the treatment of infectious diseases in man, animals, and plants, antibiotics have contributed materially to various fields of science. In the hands of qualified investigators, the antibiotics have become powerful tools for further scientific research. This is true particularly in the fields of chemistry and biology, especially in their application to agriculture, medicine, and public health. A few illustrations will suffice to indicate the extent of their contribution.

1. The knowledge of antibiotics has contributed greatly to genetics, especially microbial genetics. The development of bacterial resistance to streptomycin and other antibiotics proved to be an important genetic marker for studies on the sexual recombinations among bacteria and actinomycetes.

Crossings of parental strains with rearrangement of genetic materials are usually performed with nutritionally deficient mutants of strains that normally can grow in synthetic media with a sugar as the sole carbon source. Penicillin is usually employed to concentrate and recover the induced mutants.

2. A better understanding of biological synthesis, especially of large molecules (notably proteins, nucleic acids, and cell-wall material), can be attributed to the introduction of antibiotics. The effect of chloramphenicol on the building of the protein molecule and on amino acid incorporation established the fact that this antibiotic uncouples the synthesis of nucleic acid from that of protein (Lacks and Gros, 1959). This is also true of the effect of penicillin upon protoplast formation in *Escherichia coli*.

3. The widespread use of antibiotics has stimulated organic chemical research, and several new or very rare compounds have been discovered. Streptose, the first branched-chain sugar to be identified in a microbiological product, and streptidine, a base related to inositol, were found in streptomycin. Only three naturally occurring polyacetylenes were known before 1950, but study of the polyene antibiotics has increased the number to at least 25. Dichloroacetic acid and nitrobenzene, although well known to the organic chemist, were found in a natural product for the first time in chloramphenicol.

4. The very fact that there is relatively little cross-resistance among the various antibiotics suggests the probability that different mechanisms of antimicrobial activity are involved. This effect of antibiotics upon microbes causing infectious diseases offers the clinician a number of possibilities in the selection of chemotherapeutic agents, alone or in combination with other antibiotics or chemical substances. The whole principle involved in the preparation of polio vaccine is based on the preservation of the polioviruses, by means of antibiotics, against destruction through bacterial contamination.

5. The growth-promoting effects of antibiotics on higher animals appear to be distinctly different from those exerted by essential growth factors or vitamins. They are not so specific as are true vitamins. They involve processes heretofore scarcely recognized in nature. This stimulating effect of antibiotics has been ascribed to a disturbance of the intestinal microbial populations of the animals. A direct effect upon animal growth has also been postulated. Since antibiotics may affect adversely the bacterial population of the rumen which assists the animal in the digestion of its cellulosic food materials, care must be exercised to use antibiotics only at a certain stage of the development of the animal.

6. Human and animal semen can be preserved from bacterial attack by means of antibiotics. The same is true of the preservation of foods, especially poultry and certain vegetables. Since antibiotics do not inhibit all forms of microbial life, more than one antibiotic may be required for proper preservation. Before the food is eaten, however, the antibiotics must be destroyed by boiling, for their constant consumption in the food would tend to have certain dangerous effects upon the human body.

7. Antibiotics have introduced a new concept of microbial life in natural environments. They have added greatly to our understanding of various aspects of biology, under natural conditions. The actinomycetes have proved to be the richest source of antibiotic producers. In 1939, on the eve of the advent of antibiotics, these organisms were considered as a rather insignificant group of microbes, largely inhabiting soils, composts, and lakes. Today, because of their ability to form antibiotics, their biochemical activities and their role in nature have become problems of paramount significance.

8. Light has been thrown on numerous other scientific problems through the discovery of the potentialites of antibiotics. Such advances include the extensive use of tissue cultures in biology, a better understanding of the structure of the bacterial cell, a clearer picture of superinfections and of other problems in the field of medical research.

It is believed by some that the development of resistance to antibiotics suggests their reduced usefulness and gradual elimination. New antibiotics are introduced, only to be followed by the development of resistance to these as well. The pessimistic prophet tends to see in this the end of the antibiotic era. The optimist, however, is greatly heartened by the progress already made. He foresees the complete elimination of tuberculosis as the great enemy of man. He looks forward to the complete control of children's diseases. Such infections as undulant fever, typhoid, dysentery, cholera, plague, and even leprosy no longer hold for him the threat that they did before the advent of the antibiotics. He even looks forward to the ultimate control of such diseases as cancer, those caused by viruses, and possibly others. How soon this may come about, only the future will tell.

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Part B

Descriptions of the Various Antibiotics Produced by Actinomycetes

Introductory

Section B contains a compilation of the basic information published on the antibiotics, as well as the antiviral and the antitumor substances produced by actinomycetes.

In presenting this information, emphasis is laid upon the organism producing the antibiotic; the method of extraction of the antibiotic; its physical, chemical, and biological properties; its activity *in vivo*; toxicity; and practical utilization. A few of the most pertinent references are given for each antibiotic.

The authors have tried to make a thorough review of the literature up to July 1, 1960. After this date the coverage of the literature has been more fragmentary. Specifically, Section B contains:

1. A compilation of the information available on the various antibiotics. The substances are arranged alphabetically. Antibiotics with no names, only numbers, appear in the following order: first, those with no letters before the numbers. The first digit before which there is a break of some kind gives the order of listing, for example, *antibiotic 1-81d-1s* will be found before *antibiotic 136*. Second, the antibiotics indicated by a letter with a number, such as *antibiotic F 416*, are listed in alphabetical and numerical order. Some antibiotics, especially those which have been reported very recently, will be listed at the end under the heading "List of Additional Antibiotics." Enough will be said in that section to suggest the general nature of these substances. These substances will be indexed but will not be included in the keys and in the lists.

2. Keys which might help those who try to identify freshly isolated antibiotics. It is to be understood that these keys are not classification systems, *sensu strictu*, but they are presented here for the sole purpose of helping the investigator who is interested in characterizing antibiotic preparations.

Antibiotics are first separated on the basis of biological activity, then on the basis of chemical properties. Special attention is placed on the light-absorption spectra. Differences in what one author might consider a maximum of absorption and another a shoulder could lead to confusion. The keys lack uniformity because all antibiotics have not been described with equal care and according to the same criteria. The same substance may, therefore, be listed in the keys in more than one section. In spite of their limitations, the keys have been useful to the authors, and they hope that others will be able to use them.

3. A list of amino acids reported to be present in hydrolysates of antibiotics.

4. Lists of antibiotics active against protozoa and viruses will be found after the keys. A list of antitumor substances will also be found there.

One should note that antitumor substances, with no known activity against microorganisms, are not antibiotics as defined by the senior author. For the sake of completeness, we have tried to include such substances in this book.

The usefulness of this compilation is extended by the index found at the end of the volume, in which synonyms for the antibiotics described are listed.

In view of the rapidly accumulating information on antibiotics produced by actinomycetes, and in view of the fact that the

same or only slightly different antibiotics are often isolated in different laboratories throughout the world, there will be found a certain amount of overlapping among some of the antibiotics described in this treatise. This is true particularly of those antibiotic preparations that have not been fully described and of those that represent mixtures of closely related compounds rather than single chemical entities.

Keys to Antibiotics

KEY NO. 1

Antibiotics Which Are Active Mainly against Gram-positive Bacteria

I. Antibiotics active mainly against mycobacteria and/or actinomycetes

A. Antibiotics for which one maximum of light asborption has been reported (Each number indicates the maximum, expressed in mμ.)

1. 208 in 0.1 *N* HCl; 218 in 0.1 *N* NaOH	alboverticillin
2. 226 in water at pH 6.0	cycloserine
3. 235 in ethanol	hygroscopin A
4. 237.5 in methanol	elaiomycin
5. 260	mycomycetin
6. 260 in methanol over a wide pH range	angustmycin A
7. 268 in 0.1 *N* HCl; 280 to 282.5 in 0.1 *N* NaOH	phthiomycin viomycin
8. 270 at pH 8.4	bovinocidin
9. 303 in alcohol	pyridomycin
10. 306 in 50 per cent aqueous ethanol; 316 in 0.1 *N* HCl	amicetin

B. Antibiotics for which more than one maximum of light absorption has been reported

1. 227 and 270 in acidic solutions	tubercidin
2. 230 and 285, with end-absorption, in water	questiomycin B
3. 240, 410, and 425 in cyclohexane	questiomycin A
4. 244 and 295 to 300; ninhydrin-positive	phleomycin (base)
5. 251 and 364 in methanol	tubermycin B
6. 255 and 320 in water	grisamine
7. 256 and 365 in methanol	tubermycin A
8. 260 and 270, with weak maxima at 290, 307, 327, and 348	isomycomycin

C. Antibiotics with no light absorption or for which light absorption has not been reported

1. Neutral, white antibiotics	acetomycin longisporin
2. Acidic, colorless substance, essentially insoluble in water and chloroform	actithiazic acid
3. Heat-stable substances, probably basic and water-soluble	antiphlei antibiotics antismegmatis antibiotic nocardin
4. Reddish substance	nocardamin
5. Lytic agent	actinolysin

II. Antibiotics active against gram-positive bacteria in general

A. Antibiotics for which one maximum of light absorption has been reported

1. 203	bottromycin
2. 206	gancidin W
3. 208 in 0.1 *N* HCl	alboverticillin
4. 215 in ethanol	antibiotic E 129 (patent component B)
5. 216 in methanol	staphylomycin factor M
6. 217	megacidin
7. 220	amaromycin
8. 223	antibiotic PA 133B
9. 225	streptogramin
	picromycin
10. 226 in methanol	mikamycin A
11. 226 in water at pH 6.0	cycloserine
12. 227.5 in ethanol	neomethymycin
13. 230	miamycin
	melanosporin
	sarkomycin
14. 230–232	leucomycin
	leucomycin-like complex
	leucomycin B
	spiramycin
15. 230–235 in methanol	antibiotic E 300
16. 233 in ethanol	tertiomycin A
17. 235 in ethanol	hygroscopin A
18. 238	carbomycin
	antibiotic PA 148
19. 240	angolamycin
	hygrostatin
	proactinomycin A
20. 252 to 253	azalomycin B
	elaiophylin
21. 256 in isopropyl alcohol	borrelidin
22. 260	mycomycetin
23. 268 in 0.1 *N* HCl	phthiomycin
24. 268 to 272 in water	thermoviridin
25. 270 in methanol	xanthicin
26. 273 in water	speciomycin
27. 275	flavocidin
	antibiotic F 256
28 278 to 280	carbomycin B
	erythromycin
	antibiotic PA 108
	ristocetins

29. 280 to 282 in acid	vancomycin
30. 281 in hexane	valinomycin
31. 285 in methanol	matamycin
32. 289	erythromycin B
33. 290 to 295	oleandomycin
34. 292	erythromycin C
35. 304 in ethanol	seligocidin
36. 306 in 50 per cent aqueous ethanol	amicetin
37. 310 in 6 *N* H_2SO_4	bryamycin
38. 350	etamycin
39. 405 to 430 in dilute HCl	pluramycin A
40. 440 in methanol	nocardianin

B. Antibiotics for which two maxima of light absorption have been reported

1. 207 and 304 in methanol	staphylomycin factor S
2. 208 and 245 in ethanol	puromycin A
3. 215 and 257 to 258 in methanol	mycospocidin
4. 220 to 230 and 275 in methanol	antibiotic PA 114A
5. 222 and 293	picromycin
6. 223 to 225 and 322 in ethanol	methymycin
7. 225 and 286	narbomycin
8. 226 and 275	antibiotic PA 133A
9. 230 to 240 and 334 to 340 in 0.1 *N* HCl	abikoviromycin
10. 230 to 231 and 279 to 285 in ethanol	tertiomycin B leucomycins antibiotic 446
11. 230 to 231 and 278 to 285 in water	questiomycin B
12. 231 and 276	actinoflocin
13. 235 and 280 in 0.01 *N* NaOH	aureolic acid (Mg salt)
14. 240 and 295	proactinomycin C
15. 240 and 267 to 268 in ethanol or methanol	musarin azalomycin F
16. 240 and 310 in 0.01 *N* alcoholic sulfuric acid	celesticetin
17. 240 and 440 to 450	actinomycins
18. 243 and 320 to 325 in aqueous methanol	antibiotic F 43
19. 243 and 312	actinoleukin
20. 244 and 284	antibiotic 1-81d-1s
21. 245 and 255	antibiotic SKCC 1377 (picrate)
22. 245 and 270 to 275	proactinomycin B diazomycins
23. 245 and 440 to 450	ractinomycin A
24. 248 and 308 in 70 per cent ethanol at pH 7.5	novobiocin
25. 249 and 317 in isopropyl alcohol	antibiotic X-537-A
26. 249 and 321 in pH 7.0 phosphate buffer	amicetin B
27. 250 and 400 to 450	ractinomycin B
28. 255 and 320 in water	grisamine

29. 258 to 261 and 355 to 356 in methanol	teomycic acid
30. 260 and 305 in methanol	antibiotic PA 114B
31. 260 to 280 and 340	telomycin
32. 275 and 316	pulvomycin
33. 280 and 420 to 440 in 0.1 *N* HCl	luteomycin
34. 280.5 and 430	antibiotic L. A. 7017
35. 330 and 420 in methanol	taitomycin
36. 523 and 560 in dioxane	actinorhodin
37. 535 and 575	collinomycin
38. 536 and 567 in methanol	rhodomycin complex
39. 546 and 584 in 2 *N* NaOH	rubromycin

C. Antibiotics for which three maxima of light asborption have been reported

1. 206, 265, and 340 in 0.1 *N* HCl	gancidin A
2. 209, 260, and 305 in methanol	mikamycin B
3. 215, 255 to 257, and 430 at acid pH	antitumor antibiotic 289 antibiotic SAX 10
4. 218, 250, and 283 in ethanol	carzinophilin A
5. 218, 289, and 370 in ethanol	antibiotic X 340
6. 223, 304, and 425, in pH 7.3 phosphate buffer	rifomycin B
7. 227, 278, and 400 in ethanol	aburamycin isomer
8. 228, 258, and 425	quinocyclines
9. 230, 276, and 410 in 95 per cent ethanol	aburamycin
10. 234, 357.5, and 370 in 0.01 *N* ethanolic H_2SO_4	streptolydigin
11. 234, 540, and 580	rhodomycetin
12. 240, 280, and 305 in methanol	thiostrepton
13. 240, 410, and 425 in cyclohexane	questiomycin A
14. 240, 413 to 430, and 440 to 450	actinomycins
15. 247, 287, and 390 to 400	chrysomycin
16. 258, 420, and 471 in 95 per cent ethanol	mycorhodin
17. 317, 333, and 350 in organic solvents, pentaenes	pentaene antifungal antibiotics I and II
18. 320, 415, and 490 in n-butanol	rubidin
19. 335 to 341, 355 to 358, and 373 to 380 in organic solvents, hexaenes	flavacid mediocidin cryptocidin
20. 460 to 480, 510 to 530, and 560 to 570	litmocidin

D. Antibiotics for which more than three maxima of light absorption have been reported

1. 218, 273, 281, and 288	antibiotic PA 155A
2. 223, 286, 532, and 576 in ethanol	granaticin
3. 230, 276, 320, 330, 350, and 410 in ethanol	aburamycin
4. 230, 260, 290, and 500 in methanol	rhodomycins A and B
5. 235, 305, 525, 551, 563, and 610 in methanol	isorhodomycin A
6. 236 to 240, 266 to 270, 334 to 340, 380, 400 to 401, and 424 to 430	chartreusin coerulomycin

7. 240, 305, 385, and 461 in methanol — rifomycins
8. 269 to 270, 290, 320, 340, and 460 — heliomycin; resistomycin
9. 294, 331, 471, and 529 in alkaline ethanol — prodigiosin-like antibiotic
10. 504, 535, 542, and 575 in benzene — rhodomycins

E. Antibiotic with a progressive drop in light absorption from 210 to 450 — camphomycin

F. Antibiotics having no typical light-absorption spectra or for which light absorption data were not reported

1. Antibiotic active only against *Sarcina lutea* — sarcidin
2. Antibiotic active mainly against *Corynebacterium diphtheriae* — thermomycin
3. Antibiotic active only against respiratory-deficient staphylococci — mutomycin
4. Antibiotic active mainly against *Streptococcus hemolyticus* — totomycin
5. Antibiotics presumably active against various gram-positive bacteria
   a. Water-soluble substances
      (1) Acidic substances — streptocin; duramycin
      (2) Neutral, yellowish green substance — actinomycelin
      (3) Basic substances
         (a) Positive Sakaguchi and ninhydrin reactions — antibiotic AX 18
         (b) Positive Sakaguchi reaction, negative ninhydrin reaction — antibiotic A 116; cinnamycin
         (c) Negative Sakaguchi and ninhydrin reaction — albomycetin; amphomycin
         (d) Negative Sakaguchi reaction, positive ninhydrin reaction — trehalosamine
         (e) Unknown Sakaguchi and ninhydrin reactions — actinoidin, HCl salt
      (4) Polypeptide or proteinaceous substances — actinoxanthine; amphomycin; cinnamycin; crystallomycin; duramycin; micromonosporin; phytostreptin
      (5) Red-amber substance, brilliant yellow solution at alkaline pH — antibiotic 721
      (6) Red-brown substance. Yellow in acidic solutions, purple at alkaline pH — antibiotic SKCC 1377
   b. Substances soluble in water only at alkaline reaction

(1) pH indicator; blue at alkaline pH and red at acid pH	coelicolorin
(2) Indicator; yellow at acid pH and violet at alkaline pH	vinacetin
(3) Basic polypeptide	actinoidin
c. Essentially water-insoluble substances	
(1) Violet substances	mycetin antibiotic 1212
(2) Reddish substances	microcins pluramycin B
(3) Yellow substances	actiduins antibiotic K 125a vinacetin zaomycin
(4) Colorless or white substances	
(a) Acidic substances	antibiotic X 206 antibiotic X 464 aspartocin nigericin
(b) Neutral substance	longisporin
(c) Basic substance	mesenterin
(d) Polypeptides	aspartocin phytoactin
(e) Miscellaneous compounds, soluble in chloroform	antibiotic F 416 antibiotic J 4 exfoliatin griseomycin lustericin sulfactins
(f) Miscellaneous compounds, insoluble in chloroform	griseoflavin primycin
(g) Solubility in chloroform not known	antibiotic E 129 (component G)
(5) Color unknown	
(a) Basic compound	monamycin
(b) Acidic compound	cardicin
(c) Antibiotic soluble in chloroform	phalamycin
d. Miscellaneous compounds	
(1) Mixture of lytic enzymes	actinomycetin bacteriolytic factors
(2) Toxic pigment	antivirubin
(3) Bright yellow pigment	chromomycin A_3

KEY NO. 2

Antibiotics Which Are Active against Gram-positive and Gram-negative Bacteria

A. Antibiotics for which one maximum of light absorption has been reported	
1. 218.5	antibiotic PA 132

2. 220.5	griseoviridin
3. 226 in water at pH 6.0	cycloserine
4. 228 (very weak maxima at 380, 394, and 413)	streptozotocin
5. 235	caerulomycin
6. 243	echinomycin
7. 244.5 in ethanol	streptovaricins
8. 252 in water	antibiotic 10 CM
9. 256 in water at pH 7.0	nucleocidin
10. 259 in acid	psicofuranine
	antibiotic U 9586
11. 264 in 0.1 *M* phosphate buffer at pH 7.0	actinobolin
12. 265 in water	cellostatin
13. 265 to 275 at pH 1 to 6	xanthomycin C
14. 267.5 in 0.1 *N* HCl	puromycins
15. 268 in 0.1 *N* HCl	viomycin
16. 270 to 272 in water	homomycin
	hygromycin
17. 272	antibiotic PA 147
18. 275 in 0.1 *N* HCl	blasticidin S
19. 275 to 280	sulfocidin
20. 278 in water	chloramphenicol
21. 280 to 282 in acid	vancomycin
285 in methanol	matamycin
22. 293 in water	violacetin
23. 299 in 0.1 *N* NaOH	cellocidin
24. 300 to 320 in ethanol, more active against gram-negative bacteria than against gram-positive bacteria	enteromycin
25. 306 in 50 per cent ethanol	bamicetin
26. 313 to 314 in ethanol	azomycin
27. 365	streptocardin
28. 370 in methanol	thiomycin
29. 388 in ethanol	thiolutin
30. 500 to 530	rhodocidin

B. Antibiotics for which two maxima of light absorption have been reported

1. 215 and 315	mitomycin fraction R
2. 223 to 225 and 322 in ethanol	methymycin
3. 227 and 303 in 0.1 *N* HCl	pyridomycin
4. 230 to 240 and 334 to 340 in 0.1 *N* HCl	abikoviromycin
5. 232 and 370 in methanol	thioaurin
6. 235 and 300 to 305 in 0.03 *N* NaOH	althiomycin
7. 238 and 295	netropsin
8. 243 and 318	levomycin
9. 244 and 274	DON
10. 244.5 and 263 in ethanol	streptovaricins
11. 244 and 295 to 300	phleomycin
12. 245 and 275	diazomycins

13. 245 and 380 in methanol	streptonigrin
14. 248 and 308 in 70 per cent ethanol at pH 7.5	novobiocin
15. 250 and 320	nitrosporin
16. 257.5 and 390 to 402 in pH 6.0 phosphate buffer	xanthothricin
17. 262 and 364	ruticin
18. 265 and 362 in methanol	griseolutein A
19. 265 and 420	grisein
20. 267 and 281	mycomycin
21. 270 and 330 to 340	pleomycin
22. 270 and 370	oxytetracycline
23. 281 to 283 and 342 to 344 in methanol	griseolutein B
24. 288 and 460 in ethanol	xanthomycin A
25. 290 and 330 at pH 1 or 6	xanthomycin B
26. 498 and 532 in n-butanol	violarin

C. Antibiotics for which three maxima of light absorption have been reported

1. 215, 316 to 318, and 530 in water	mitomycin A and B
2. 216, 360, and 560	mitomycin C
3. 218, 289, and 370 in ethanol	antibiotic X 340
4. 227, 260, and 370 in 0.1 *N* HCl	bromtetracycline
5. 227, 271, and 304	antibiotic 7, 080 R. P.
6. 230, 275, and 367.5 in water	chlortetracycline
7. 239, 270 to 280, and 340 in pH 7.8 phosphate buffer	fervenulin
8. 240, 278, and 384 in 0.1 *N* HCl	cyanomycin
9. 248, 312, and 388	aureothricin
10. 250, 303, and 390 in ethanol	holomycin
11. 250, 311, and 388 in ethanol	thiolutin
12. 288, 301, and 316, tetraene	tennecetin
13. 292, 308, and 320, tetraene	endomycin A
14. 320, 415, and 490 in n-butanol	rubidin
15. 338, 359, and 380, hexaene	endomycin B

D. Antibiotics for which four maxima of light absorption have been reported

1. 207, 237, 286, and 343	mitomycin fraction Y
2. 222, 266, 286, and 369 in methanol	nybomycin
3. 228, 258, 288, and 427	aklavin
4. 235, 269, 298.5, and 365 in methanol	tetracycline
5. 240, 255, 305, and 375	albofungin

E. Antibiotics which exhibit either only end-absorption of ultraviolet light, or no typical light absorption, or for which no light-absorption data have been reported

1. Red substances	antibiotic of Chandrasekhar cladomycin
2. Indicator; red in alkaline solution, yellow in acid solution	nocardorubin
3. Yellow substances	
a. Acidic	antibiotic of Rolland
b. Basic, water-insoluble	raisnomycin

4. Essentially white substances, basic, water-soluble
   a. Positive Sakaguchi reaction; negative ninhydrin reaction
      streptomycin group — hydroxystreptomycin
      streptomycin — dihydrostreptomycin
      pseudostreptomycin — dihydrodesoxystreptomycin
      mannosidostreptomycin
   b. Positive Sakaguchi reaction, positive ninhydrin reaction — antibiotic 1943; eurimycin
   c. Positive Sakaguchi reaction; ninhydrin reaction not reported — antibiotic A 6; antibiotic A 20
   d. Negative Sakaguchi and ninhydrin reactions — antibiotic 587/13; flaveolin; fuscomycin; roseocitrin B; roseomycin
   e. Negative Sakaguchi reaction; positive ninhydrin reaction
      (1) Substance yielding at least one amino acid upon hydrolysis
      streptothricin group — luridin
      actinorubin — mycothricin
      antibiotic 136 — pleocidins
      antibiotic 156 — racemomycins
      antibiotic EI_5 — roseothricin
      evericin — streptin
      geomycins — streptolins
      grasseriomycin — streptothricin
      grizin — desertomycin?
      lavendulin — antibiotic GB/229?
      (2) Substances yielding no amino acids upon hydrolysis; amino sugar compounds
      neomycins — monomycin
      kanamycins — paromomycin
      catenulin — novomycin?
      hydroxymycin — trehalosamine
   f. Unknown ninhydrin and Sakaguchi reactions
      antibiotic GB/229? — neonocardin
      antibiotic of Mukherjee — virusin 1609
      hygromycin B
5. Polypeptides or proteinaceous substances
      actinin — cephalomycin
      antibiotic of Sackmann — actinomycetin
      echinomycin — bacteriolytic factors
      neocide

6. Basic substance soluble in water at acid pH, insoluble in chloroform	phagolessin
7. Neutral or weakly basic compound, soluble in chloroform	phagostatin
8. Benzenoid-type compound containing no nitrogen	ramnacin
9. Contains nitrogen, water-soluble substance	desertomycin totomycin
10. No data to permit classification	miramycin

KEY NO. 3

Antibiotics Which Have Antifungal Activity[1]

I. Antibiotics active not only against fungi but also against gram-positive bacteria	
A. Active against *M. tuberculosis* var. *hominis* but not against other bacteria	
1. Weakly basic substance, light-absorption maxima in water at 230 and 277, and in 0.1 to 0.05 N HCl at 235 to 236 and 273	toyocamycin unamycin B
2. Oil, light-absorption maximum at 235 in ethanol	hygroscopin A
B. Active against strains of *Nocardia asteroides*, but not against other bacteria or streptomycetes	
1. Light-absorption maxima at 263 and 268 soluble with difficulty in water	flavofungin
2. Basic, water-soluble substance	eulicin
C. Active against strains of *Nocardia* and *Streptomyces*, but not against bacteria	
1. Light-absorption maximum at 302 in methanol	eumycetin
D. Active against gram-positive bacteria *stricto sensu*	
1. Substances for which one maximum of light absorption has been reported, as follows:	
a. 230 in methanol	melanosporin
b. 240, with shoulder at 255 to 270; yellow, water-insoluble substance	hygrostatin
c. 270 in methanol; yellow substance	xanthicin
d. 275 in methanol	antibiotic F 256
e. 304 in ethanol	seligocidin
f. 310, with strong end-absorption below 250 in 6 *N* H_2SO_4; sulfur-containing polypeptide	bryamycin
2. Substances for which two maxima of light absorption have been reported	
a. 215 and 257 to 258 in methanol	mycospocidin
b. 230 and 285 in water	questiomycin B
c. 240 and 267 to 268 in ethanol or methanol	azalomycin F musarin

[1] In this key, actinomycetes, including mycobacteria, are considered as gram-positive bacteria.

d. 243 and 320 to 325 in methanol	antibiotic F 43
e. 244 and 284 in methanol	antibiotic 1-81d-1s
f. 245 and 275	diazomycins
g. 245 to 250 and 400 to 450	ractinomycins
3. Substances for which three maxima of light absorption have been reported	
a. Pentaenes; about 317, 333, and 350	pentaene antifungal antibiotics I and II
b. Hexaenes; about 338, 357, and 378	cryptocidin flavacid mediocidin
c. 240, 410, and 425 in cyclohexane	questiomycin A
4. Substances which have been reported to have only end-absorption in ultraviolet light	antibiotic F 416 lustericin
5. Substances giving a red color at alkaline reaction	
a. Heating with NaOH produces red color and a basic gas	camphomycin antibiotic 1-81d-1s
b. Greenish yellow; active mainly against phages; gives inactive red solutions at alkaline reaction	chrysomycin
6. Miscellaneous substances	
a. Yellowish red to reddish violet substances	ractinomycins microcins
b. Nonsulfur-containing polypeptide	phytoactin phytostreptin
c. Acidic substances	nigericin cardicin
d. Acidic polypeptide	duramycin

II. Antibiotics active not only against fungi, but also against gram-positive and gram-negative bacteria

A. Substances for which one light-absorption maximum has been reported	
1. 235, with shoulders at 285 and 295	caerulomycin
2. 244, with a shoulder at 263 and a plateau at 316; bright orange materials; yellow in acid solution, red-amber in alkaline solution	streptovaricins
3. 265 in water	cellostatin
4. 275 in 0.1 *N* HCl or at 266 to 270 in 0.1 *N* NaOH	blasticidin S
5. 275 to 280 in ethanol; contains sulfur	sulfocidin
6. 293 in water	violacetin
7. 388 in ethanol	thiolutin
B. Substances for which two light-absorption maxima have been reported	
1. 232 and 370 in methanol; contains sulfur	thioaurin
2. 244 and 274	DON
3. 266 and 285 in ethanol; water-insoluble base	nybomycin
4. 267 and 280, with an inflection at 256; very unstable acid	mycomycin

C. Substances for which three light-absorption maxima have been reported
1. 239, 270 to 280, and 340 in pH 7.8 phosphate buffer — fervenulin
2. 248 to 250, 311 to 312, and 388 in ethanol — aureothricin; thiolutin
3. 288 to 292, 300 to 308, and 315 to 320; tetraenes — endomycin A; tennecetin
4. 338, 359, and 380; hexaene — endomycin B

D. Substances for which more than three light-absorption maxima have been reported
1. 228, 258, 288, and 427; reddish purple color with H_2SO_4 — aklavin
2. 240, 255, 305, and 375; yellow, water-insoluble substance — albofungin

E. Water-soluble substances with no specific light-absorption spectra, but often with end-absorption of ultraviolet light

actinorubin	grizin
antibiotics 136, A 20, GB/229, and 587/13	hygromycin B
	mycothricins
cephalomycin	streptothricin
grasseriomycin	virusin 1609

F. Miscellaneous substances
1. Basic substance, soluble in water, yellow in acid solution, reddish in alkaline solution — flaveolin
2. Water-insoluble substance; can be autoclaved from pH 2 to 10 — ramnacin

III. Antibiotics active mainly against fungi, and with little or no activity against bacteria

A. Substances for which visible and/or ultraviolet light absorption has been reported
1. Tetraenes: light-absorption maxima at about 290 to 292, 300 to 305, and 317 to 320

amphotericin A	nystatin
antibiotic PA 86	pimaricin
antibiotic PA 166	protocidin
antifungal antibiotic 7071 R. P.	rimocidin
antifungal antibiotic J 4B	sistomycosin
antimycoin	tetraene antifungal antibiotic
chromin	tetrin
endomycin A	unamycin
etruscomycin	

2. Pentaenes: light-absorption maxima at about 317 to 324, 330 to 340, and 349 to 358

aliomycin	fungichromatin
antibiotic PA 153	lagosin
cabicidin	moldcidin A
eurocidin	pentaene antifungal antibiotics I and II
filipin	
fungichromin	pentamycin

3. Hexaenes: light-absorption maxima at about 335 to 338, 355 to 359, and 373 to 380
   - cryptocidin
   - endomycin B
   - flavacid
   - mediocidin
   - mycelin-IMO
4. Heptaenes: light-absorption maxima at about 358 to 366, 377 to 388, and 399 to 410
   - amphotericin B
   - antibiotic 26/1
   - antibiotic AYF
   - antibiotic PA 150
   - antifungal antibiotic 757
   - antifungal antibiotic A 228
   - antifungal antibiotic of Rao and Uma
   - antifungal heptaene F 17C
   - ascosin
   - aureofacin
   - ayfactin
   - candicidin
   - candidin
   - candimycin
   - grubilin
   - perimycin
   - trichomycin
5. Nonpolyenic substances for which one light-absorption maximum has been reported, as follows:
   - a. 216 — blasticidin A
   - b. 235 in ethanol — hygroscopin A
   - c. 237.5 in methanol — elaiomycin
   - d. 250.5 in pH 7.0 phosphate buffer — azaserine
   - e. 251 in methanol — flavensomycin
   - f. 258 — niger factor
   - g. 287 — cycloheximide
   - h. 290 — fermicidin
   - i. 302 in methanol — eumycetin
   - j. 320 — moldin
6. Nonpolyenic substances for which two light-absorption maxima have been reported
   - a. 212 and 260 — antifungal antibiotic J 4A
   - b. 225 and 232 in ethanol — oligomycins
   - c. about 225 to 228 and 320 to 330 — antimycins, blastmycins
   - d. 227 and 337 — vulgarin
   - e. 230 and 280 in water — monilin
   - f. 232 and 291 in methanol — streptimidone
   - g. 233 to 236 and 272 to 273 in 0.05 N HCl — unamycin B, vengicide
   - h. 242 and 292 in methanol — fradicin
   - i. 245 and 285 — humidin
   - j. 254 to 255 and 345 in methanol — mycolutein, antibiotic 2814K
   - k. 263 and 268 — flavofungin
   - l. 270 and 350 in methanol — alomycin

7. Nonpolyenic substances for which three or more light-absorption maxima have been reported	
a. 210, 263, and 363	mycoticin
b. 224, 277, and 283 in ethanol	anisomycin
c. 243, 294, 335, 355, and 373 in methanol	fradicin
d. 272, 372, and 440 to 445 in methanol	1,6-dihydroxyphenazine
8. Substances reported to have only end-absorption in ultraviolet light	niromycins streptovitacins
9. Substances reported to have no absorption in ultraviolet light	
amidomycin	cerevioccidin
carzinocidin	niromycins
10. Substances for which light absorption has not been reported	
a. Soluble in water	
bulging factor	eulicin
antibiotic A 67	
b. Insoluble in water	
(1) Soluble in chloroform	
antibiotic 30-10	mycelin
cacaomycetin	phaeofacin
(2) Insoluble in chloroform	actinone rotaventin

KEY NO. 4

Substances, Produced by Actinomycetes, Which Have Been Reported to Absorb Ultraviolet and/or Visible Light, and Which Have Not Been Reported to be Active against Bacteria and/or Fungi

I. Substances for which one maximum of light absorption has been reported	
A. 206	gancidin W
B. 216	lenamycin
C. 217 in methanol	antitumor substance 1418A1
D. 233 in ethanol	hygroscopin B
E. 259 in 0.01 *N* acid or 261 in 0.01 *N* base	psicofuranine
F. 260 to 270	melanomycin
G. 260 in water	angustmycin C
H. 264	nonactin
I. 274 in 0.1 *N* HCl	isokojic acid
J. 330 in ethanol	inactone
II. Substances for which more than one maximum of light absorption has been reported	
A. 225 and 337.5 in alkaline aqueous solution	antitumor substance 1418A1
B. 230 to 240 and 334 to 340 in 0.1 *N* HCl and 244 to 246 and 280 to 291 in 0.1 *N* NaOH	abikoviromycin

C. 235 and 300 in methanol	phagocidin
D. 238 and 335 in acidic water	virocidin
E. 240 and 285 in pH 6.0 phosphate buffer	carimbose
F. 242 and 274 in pH 7.0 phosphate buffer	alazopeptin
G. 244 and 274	DON
H. 245 and 285	antibiotic PA 128
I. 285 and 320	antibiotic 6270

LIST NO. 1

Antibiotics Which Have Been Reported to Have Antiprotozoan Activity

I. Substances active against only protozoa

antibiotic PA 128
protomycin

II. Substances active against protozoa and fungi

aliomycin	fradicin
anisomycin	humidin
antifungal antibiotic A 228	moldcidin A
azalomycin F	nystatin
candimycin	pimaricin
endomycins	ractinomycins
etruscomycin	rimocidin
eurocidin	rotaventin
fervenulin	streptimidone
fermicidin	streptovitacins
filipin	trichomycin

III. Substances active against protozoa and gram-negative and gram-positive bacteria

abikoviromycin	griseolutein B
actinobolin	neomycin
antibiotic 7,080 R. P.	netropsin
antibiotic PA 132	nucleocidin
azomycin	oxytetracycline
cycloserine	paromomycin
echinomycin	phagolessin
endomycins	puromycins
fervenulin	sarkomycin
hygromycin	tetracycline
geomycins	xanthomycins

IV. Substances active against protozoa and gram-positive bacteria

aburamycin isomer	azalomycin F
acetomycin	borrelidin
amphomycin	carbomycins
angolamycin	erythromycins
aureolic acid	etamycin

granaticin
oleandomycin
prodigiosin-like antibiotic
ractinomycins
spiramycin
streptocin
valinomycin
vancomycin

LIST NO. 2

Antibiotics Which Have Been Reported to Have Antiviral Activity

I. Antibiotics active only against viruses
- achromoviromycin
- ehrlichin
- hygroscopin B
- myxoviromycin
- noformicin
- phagocidin
- phagomycin
- virocidin

II. Antibiotics active against viruses and fungi
- antimycins
- fermicidin
- hygroscopin A
- niger factor
- niromycins

III. Antibiotics active against viruses, fungi, and gram-positive and gram-negative bacteria
- aklavin
- cephalomycin
- grasseriomycin
- nybomycin
- violacetin
- virusin 1609

IV. Antibiotics active against viruses, fungi, and gram-positive bacteria
- antibiotic 1-81d-1s
- antibiotic F 43
- antibiotic F 256
- antibiotic F 416
- cardicin
- chrysomycin
- toyocamycin

V. Antibiotics active against viruses and gram-positive and gram-negative bacteria
- bromtetracycline
- chloramphenicol
- chlortetracycline
- echinomycin
- enteromycin
- griseoviridin
- hygromycin
- luridin
- mitomycin C
- oxytetracycline
- phagolessin
- phagostatin
- tetracycline
- violarin

VI. Antibiotics active against viruses and gram-positive bacteria
- actinomycetin
- antibiotic 1212
- antibiotic E 300
- antivirubin
- carbomycins
- chartreusin
- chromomycins
- coerulomycin
- erythromycins
- flavocidin
- heliomycin
- leucomycin
- oleandomycin
- primycin
- spiramycin
- thiostrepton

LIST NO. 3

Substances Which Have Been Reported to Have Activity against Tumors

I. Substances active mainly against tumors

alazopeptin
antibiotic 6270
antitumor substance 1418A1
antitumor antibiotic E 73
carcinomycin
caryomycin
DON
gancidin W
hygroscopin B
lenamycin
melanomycin
psicofuranine
raromycin

II. Substances active against tumors and fungi

aliomycin
antimycins
aureothricin
azaserine
candimycin
carzinocidin
cellostatin
diazomycins
filipin
hygroscopin A
pentamycin
rimocidin
streptovitacins
sulfocidin
toyocamycin

III. Substances active against tumors and gram-positive and gram-negative bacteria

actinin
actinobolin
aureothricin
antibiotic U 9586
azomycin
cellostatin
desertomycin
echinomycin
griseolutein B
mitomycins
neocide
netropsin
psicofuranine
puromycins
sulfocidin
streptonigrin

IV. Substances active against tumors and gram-positive bacteria

aburamycin and isomer
actinoflocin
actinoleukin
actinomycins
actinoxanthine
amicetin
antitumor antibiotic 289
borrelidin
carzinophilin A
cellocidin
chromomycins
diazomycins
etamycin
gancidin
hygroscopin A
mutomycin
netropsin
pluramycins
ractinomycins
sarkomycin
toyocamycin
tubercidin

LIST NO. 4

Amino Acids Identified (or Suspected to Be Present) in the Hydrolysates of Antibiotics for Which Abstracts Have Been Written

Alanine
- alazopeptin
- antibiotic 6270
- bryamycin
- cephalomycin
- geomycin
- matamycin
- melanomycin
- neocide
- phalamycin
- phytoactin
- phytostreptin
- telomycin
- thiostrepton
- vinactins A and B

L-Alanine
- echinomycin
- etamycin

Alanine (N-methyl)
- actinomycins

Alanine (β-(2-thiazole)-β-)
- bottromycin

α-Aminobutyric acid
- staphylomycin

α-Amino-β-phenylbutyric acid
- bottromycin

Arginine
- cephalomycin
- cinnamycin
- matamycin
- melanomycin
- neocide
- phytoactin
- phytostreptin

Aspartic acid
- amphomycin
- aspartocin

Aspartic Acid—*continued*
- cephalomycin
- cinnamycin
- duramycin
- geomycin
- neocide
- telomycin
- thiostrepton
- vancomycin
- vinactins A and B

Cysteine
- bryamycin
- griseoviridin
- matamycin
- neocide

Cystine
- bryamycin
- carzinocidin
- neocide
- thiostrepton

α,β-Diaminopropionic acid
- viomycin

Glutamic acid
- alazopeptin
- carzinocidin
- cephalomycin
- duramycin
- geomycin
- grisein
- melanomycin
- neocide
- noformicin
- thiostrepton
- vinactins A and B

lycine
- amphomycin
- antimycins
- aspartocin

Glycine—*continued*
- bottromycin
- bryamycin
- carzinocidin
- cephalomycin
- cinnamycin
- duramycin
- geomycin
- matamycin
- melanomycin
- mikamycin
- mycospocidin
- neocide
- phalamycin
- phytoactin
- phytostreptin
- telomycin
- thiostrepton
- vinactins A, B, and C

Histidine
- melanomycin

Isoleucine
- bryamycin
- phalamycin
- phytoactin
- phytostreptin
- thiostrepton

Isoleucine (D-allo-)
- actinomycins

Isoleucine (methyl-)
- actinomycins
- duramycin

Lanthionine
- duramycin

Lanthionine (meso-)
- cinnamycin

Leucine
- antibiotic 156
- cephalomycin
- melanomycin
- phytoactin
- phytostreptin
- thiostrepton

D-Leucine
- etamycin

Leucine (dimethyl-)
- antibiotic 6270
- etamycin (L-β,N-)

Lysine
- antibiotic 156
- carzinocidin
- neocide
- thiostrepton
- vinactins A and B

β-Lysine
- geomycin
- mycothricin
- racemomycin
- roseothricin
- streptolins
- streptothricin
- viomycin

Norvaline
- staphylomycin

Ornithine
- duramycin
- grisein

Phenylalanine
- cephalomycin
- cinnamycin
- duramycin
- melanomycin
- staphylomycin

Phenylalanine (3′-desoxy 3′-*p*-methoxy-)
- puromycin

Picolinic acid (3 hydroxy-)
- etamycin

Proline
- amphomycin
- antibiotic 156
- cinnamycin
- duramycin
- melanomycin
- neocide
- phytoactin
- phytostreptin
- staphylomycin
- thiostrepton

L-Proline
- actinomycins
- aspartocin
- mikamycin

D-Proline (allohydroxy-)
- etamycin

Proline (4-hydroxy-)
- actinomycins

Proline (4-keto-)
- actinomycins

Sarcosine
- actinomycins
- etamycin
- vinactin C

Sarcosine (L-α-phenyl-)
- etamycin

Serine
- antibiotic 156
- antibiotic 6270
- geomycin
- grisein
- griseoviridin
- matamycin
- mycothricin
- neocide
- phytoactin

Serine—*continued*
- phytostreptin
- vinactins A, B, and C

D-Serine
- echinomycin

L-Serine
- viomycin

Streptolidine
- geomycin
- racemomycin
- roseothricin
- streptolins
- streptothricin

Threonine
- bryamycin
- cephalomycin
- geomycin
- staphylomycin
- telomycin
- thiostrepton

L-Threonine
- actinomycins
- etamycin
- pyridomycin

Tryptophan
- thiostrepton

Tyrosine
- cephalomycin

Valine
- amphomycin
- antibiotic 156
- aspartocin
- bottromycin
- cephalomycin
- cinnamycin
- duramycin

Valine—*continued*
phytoactin
phytostreptin
thiostrepton
D-Valine
actinomycins
amidomycin
D-Valine—*continued*
valinomycin
L-Valine
valinomycin
L-Valine (N-methyl-)
actinomycins
echinomycin

Descriptions of Antibiotics

Abikoviromycin

Produced by: Streptomyces abikoensum (1, 2), also known as *S. abikoensis* (3). This culture also produces a heptaene antifungal substance and viomycin (7). *S. rubescens* (1, 2); *S. reticuli* var. *latumcidicus* (5), said to resemble *S. abikoensum* (6); *Streptomyces* sp. (4).

Synonyms: Latumcidin (6), virocidin (?).

Method of extraction: I. Broth-filtrate treated with ethyl acetate at neutrality. Extract concentrated and chromatographed on alumina after addition of petroleum ether; column developed with petroleum ether, then with ethyl acetate. Exceedingly unstable; glucose must be added before the solvent can be removed *in vacuo* (1, 2). II. All procedures are carried out in the cold under N_2. Broth is adjusted to pH 8, and extracted with methyl isobutyl ketone. Back-extraction into water at pH 2.0. Extraction from water with ether at pH 8, and re-extraction into water at pH 2.0. Extraction of aqueous layer with methyl isobutyl ketone at pH 8, followed by addition of concentrated H_2SO_4 to precipitate the active substance. Re-crystallized from methanol or ether (5).

Chemical and physical properties: Unstable basic substance (5). Sublimes during freeze-drying (1, 2). The sulfate forms white needles, m.p. 140–140.5°C (decomposition) (5) or 120–125°C (decomposition) (6). Soluble in water and methanol. Slightly soluble in ethanol, butanol, and acetone. Insoluble in other organic solvents (5). Ultraviolet absorption spectrum maxima at 230 to 240 mμ and 334 to 340 mμ in 0.1 *N* HCl; or at 244 to 246 mμ and 280 to 291 mμ in 0.1 *N* NaOH (6). Infrared spectra given in ref. 6. $[\alpha]_D^{21} = +148.9°$ (c = 0.1 per cent in 0.1 *N* NaOH). Positive diazo, Bayer, and bromine tests (5). Conflicting reports on Molisch and Tollen silver mirror tests. Negative $FeCl_3$, Fehling, Ehrlich, Sakaguchi, ninhydrin, biuret, and xanthoproteic tests, and tests for primary, secondary, and amyl amines. Turns red after decomposition (1, 2, 5). Most unstable at pH 4 to 7 (5) and less stable in water and ethanol than in other solvents. Readily auto-oxidized (1, 2). $C_{11}H_{13}O_2NH_2SO_4$. C = 46.6%; H = 5.13%; O = 31.18%; N = 4.99%; S = 12.10% (5).

Biological activity: Active on western and eastern equine encephalomyelitis viruses in contact tests, but not on Venezuela equine encephalomyelitis or on Japanese B encephalitis viruses (1, 2, 6). Weakly active on bacteria and mycobacteria. Conflicting reports on antifungal activity (1, 2, 5). Active on *Tetrahymena* at 5 μg per ml (5).

Toxicity: LD_{50} (mice) 8 mg per kg (1, 2) or 17.3 mg per kg (5) intravenously, and 100 mg per kg subcutaneously (1, 2).

References:

1. Umezawa, H. *et al.* Japan. Med. J. **4:** 331–346, 1951.
2. Umezawa, H. *et al.* J. Antibiotics (Japan) **5:** 469–476, 1952.
3. Okami, Y. J. Antibiotics (Japan) **5:** 477–480, 1952.
4. Umezawa, H. *et al.* Japan J. Med. Sci. & Biol. **6:** 261–268, 1953.
5. Sakagami, Y. *et al.* J. Antibiotics (Japan) **11A:** 6–13, 1958.
6. Sakagami, Y. *et al.* J. Antibiotics (Japan) **11A:** 231–232, 1958.
7. Arai, T. *et al.* Antibiotics & Chemotherapy **7:** 435–442, 1957.

Aburamycin

Produced by: *Streptomyces aburaviensis.*

Synonym: Similar to aureolic acid, but differs in chemical tests.

Method of extraction: Broth adjusted to pH 9.5 and filtered. Filtrate extracted at pH 2.0 with ethyl acetate. Ethyl acetate back-extracted with water at pH 9.0. Chromatography on alumina and development with 80 per cent methanol. Active fractions concentrated *in vacuo*. Addition of water to concentrate precipitated aburamycin. Re-crystallization from aqueous ethanol.

Chemical and physical properties: Weakly acidic substance. Yellow crystals; m.p. 163–165°C. Very soluble in ethanol, acetone, ethyl acetate, butyl acetate, butanol, and chloroform. Insoluble in water, ether, benzene, ligroin, and petroleum ether. C = 55.57%; H = 7.54%; O = 36.89%. No N, S, or halogen. Positive Molisch, Fehling, Benedict, Tollen, Seliwanoff, anthrone, and orcin tests. Negative biuret, xanthoproteic, ninhydrin, Tollen phloroglucine, $FeCl_3$, Folin, and tyrosine reactions. $[\alpha]_D^{20} = +24.56°$ (c = 1 per cent in methanol). Ultraviolet absorption spectrum maxima at 230, 276, and 410 mμ with weak maxima at 320, 330, and 350 mμ (95 per cent ethanol); or 229, 276, and 410 mμ with weak maxima at 316 and 330 mμ (0.01 N HCl); or 234, 278, 316, and 410 mμ (0.01 N NaOH). Infrared spectrum given in reference 1. More stable at alkaline than acid pH.

Biological activity: Active on gram-positive

bacteria and *M. phlei*. Not active on other mycobacteria, gram-negative bacteria, or *C. albicans*. Is 64 times more active on *Staph. aureus* at pH 6.0 than at pH 8.5. Some antitumor activity in mice against the ascitic form of Ehrlich carcinoma and the solid form of Crocker sarcoma 180.

Toxicity: LD_{50} (mice) 2 mg per kg intravenously, and 2.5 mg per kg subcutaneously. Not well absorbed from intestinal tract.

Reference: 1. Nishimura, H. *et al.* J. Antibiotics (Japan) **10A:** 205–212, 1957.

Aburamycin Isomer

Produced by: *Streptomyces* sp. differing from *S. aburaviensis*.

Synonym: Antibiotic M5-18903. Both aburamycin and its isomer have similarities with aureolic acid.

Method of extraction: I. Broth-filtrate extracted with chloroform at pH 7.2 to 7.5. Precipitated from extract by addition of petroleum ether. Chromatographed on alumina with chloroform as solvent and developer. Eluted with 5 per cent ethanol in chloroform. Active fractions concentrated under reduced pressure and precipitated with petroleum ether. Crystallized from chloroform on addition of petroleum ether. II. Same as that for aburamycin.

Chemical and physical properties: Weakly acidic substance. Yellow crystals; m.p. 169–171°C. Soluble in chloroform, acetone, pyridine, ethyl acetate, dimethylformamide, ethanol, and methanol. Insoluble in water and petroleum ether. Ultraviolet absorption spectrum maxima at 227 mμ ($E^{1\%}_{1\,cm}$ 200 ± 5), 278 mμ ($E^{1\%}_{1\,cm}$ 400 ± 10), and 400 mμ. Weak maxima at 304, 317 ($E^{1\%}_{1\,cm}$ 60 ± 2), and 330 mμ (ethanol). Infrared spectrum given in reference 1. $[\alpha]^{25}_{D} = -29°$ (c = 0.5 per cent in methanol). $pK'_a = 7.1$. C = 56.32%; H = 7.44%; O = 35.24%. No N, S, or halogen. Molecular weight, 1295. Paper chromatographic behavior is indistinguishable from aburamycin. Can be hydrogenated to yield a biologically active compound which has similar ultraviolet spectra in alkaline and acid solution to the hydrogenated product from aureolic acid. Acetylation of the hydrogenated product destroys the biological activity. The acetyl derivative has m.p. at 205–207°C.

Biological activity: Active on gram-positive bacteria, including mycobacteria. Not active on gram-negative bacteria. Not active on bacteria *in vivo*. Some activity on *Endamoeba histolytica in vivo*. Reduces the worm burden in mice infected with *Syphacia obvelata*. Some protection against leukemia P-1534 in mice, but no activity on sarcoma 180.

Toxicity: LD_{50} (mice) 2 mg per kg intravenously. Mice tolerate a single oral dose of 80 mg per kg. The antibiotic was shown to be unabsorbed from the injection site.

Reference: 1. Gale, R. M. *et al.* Antibiotics Ann. 489–492. 1958–1959.

Acetomycin

Produced by: Streptomyces ramulosis (1).

Method of extraction: Broth filtered with a filter-aid, and filtrate extracted with ethyl acetate. Extract concentrated *in vacuo*. Addition of petroleum ether to the residue precipitated acetomycin. Crystallized in the cold from hot methanol (1).

Chemical and physical properties: Neutral, saturated, ketoacetoxy lactone, with three methyl groups. Colorless, rough rod-like crystals. Sublimes at 70°C; m.p. 115–116°C. $[\alpha]_D = -167°$ (c = 1.47 per cent in ethanol). $C_{10}H_{14}O_5$: C = 55.95%; H = 6.65%; O = 37.31%. No ultra violet absorption. Infrared spectrum given in reference 1. Forms a mono-2,4-nitrophenylhydrazone: dark yellow crystalline leaves; m.p. 205–208°C. Positive iodoform reaction. Negative $FeCl_3$ test. Structural formula given in Chapter 6.

Biological activity: Weak activity on gram-positive and gram-negative bacteria (100 μg per ml); moderately active on *M. tuberculosis* H37Rv (10 μg per ml), *Trichomonas foetus* (25 μg per ml), and *E. histolytica* (70 μg per ml) (1).

Toxicity: LD_{50} (mice) 100 mg per kg subcutaneously (1).

References:

1. Ettlinger, L. *et al.* Helv. Chim. Acta **41:** 216–219, 1958.
2. Keller-Schierlein, W. *et al.* Helv. Chim. Acta **41:** 220–228, 1958.

Achromoviromycin

Produced by: Streptomyces achromogenes.

Remarks: The same strain produces sarcidin.

Method of extraction: Extraction with ethyl acetate at pH 2.0, concentration *in vacuo* at 40°C. Freeze drying after addition of 2.0 per cent $NaHCO_3$ to pH 7.5

Chemical and physical properties: Negative Molisch, Tollen, ninhydrin, Sakaguchi, Millon, Pauly, and $FeCl_3$ tests.

Biological activity: Active *in vitro* and *in vivo* against Japanese B encephalitis virus. Not active against western equine encephalitis virus.

Toxicity: 20 mg per mouse of the crude substance

did not induce any toxic signs when injected subcutaneously.

Reference: 1. Umezawa, H. *et al.* Japan. J. Med. Sci. & Biol. **6:** 261–268, 1953.

Actiduins

Produced by: Streptomyces sp.

Method of extraction: Isolated from broth-filtrates and mycelial extracts by acid, or complex precipitation, solvent extraction, or absorption techniques. Purified by solvent precipitation, chromatography, and countercurrent distribution.

Chemical and physical properties: Complex composed of six components, very closely related. Yellow to reddish substances. Darken at 280°C, but do not melt up to 350°C. Insoluble in water and slightly soluble in butanol, ethanol, pyridine, acetic acid, cyclohexane, and dimethylformamide. Certain variation exists among the components as to solubility in acetone, methanol, and chloroform. Soluble in strong mineral acids, and are precipitated on dilution. Most stable at acid and neutral pH; unstable to alkali. Not readily diffusible. *Reineckates:* Soluble in acetone. Rf (butanol-acetic acid-water, 62:12:26) 0.95 (tail). Infrared data given on components II, III, and VI in reference 1. All give a yellowish fluorescence in organic solvents; is less intense in aqueous solvents. Contain C, H, N, and S, and in one case, Cl. Acid or alkaline hydrolysates contain a fluorescent acidic product and a ninhydrin-positive material composed of a neutral and an acidic fraction.

Biological activity: Active on gram-positive bacteria. Not active on gram-negative bacteria or mycobacteria.

Reference: 1. Burton, H. S. Chem. & Ind. 442–443, 1955.

Actinin

Produced by: Streptothrix (*Streptomyces*) *felis.*

Synonym: Mycetin.

Remarks: Organism isolated from a granulomatous lesion of a cat; pathogenic for various animals.

Method of extraction: No data.

Chemical and physical properties: Basic polypeptide.

Biological activity: Active (in mice) on Ehrlich adenocarcinoma when administered as the culture fluid, destroying the tumor completely. DeAngelis (1, 2) claims to have first demonstrated, in 1936, the cancerolytic properties of this culture, and he considers it to be the first antitumor antibiotic. Active on gram-positive and gram-negative bacteria.

Toxicity: Said to be nontoxic when given in doses which destroy tumors.

Utilization: Is said to have caused complete regression of certain types of cancer in man.

References:

1. DeAngelis, G. Oncologia **2:** 43–62, 1949.
2. DeAngelis, G. Ateneo parmense **28:** 248–260, 1957.

Actinobolin

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate adsorbed on Darco G-60 at pH 4.0. Eluted with 30 to 40 per cent aqueous acetone. Eluates adjusted to pH 3.5 and concentrated *in vacuo*. Chromatographed on Decalso (at pH 6.0) and eluted with 5 per cent aqueous acetic acid containing 10 per cent ethanol. Active fractions adsorbed on a Darco G-60-Celite 545 mixture (1:1) and eluted with 20 per cent aqueous acetone. Active fraction adjusted to pH 3.5, concentrated *in vacuo*, and mixed with 0.02 M cupferron ($C_6H_5N(NO)ONH_4$) in 50:50 n-butanol-chloroform to remove iron. Aqueous layer washed with chloroform, concentrated *in vacuo* at pH 3.2, then freeze dried. Purification by salt conversion. Sulfate crystallized from water-ethanol (25:45). Occasional difficulties were encountered because of the tendency of actinobolin to form complexes with aluminum (and other Group III elements). Aluminum was removed by oxine precipitation or preferential adsorption on Dowex 50 columns (2).

Chemical and physical properties: Amphoteric substance. Base is amorphous, white, fluffy powder. Hydrophilic; very soluble in water. Could not be extracted from aqueous solution with any common water-immiscible organic solvent. Basic: $pK'_a = 7.5$ and a weakly acidic (enolic) $pK'_a = 8.8$. Gives a purple color with ninhydrin, red-orange with the Pauly diazo reagent, a red color with $FeCl_3$. Positive $KMnO_4$, Fehling, Folin-Ciocalteu, and iodoform tests. Negative Molisch, Ehrlich (dimethylaminobenzaldehyde), and Elson-Morgan tests. No reactive carbonyl. Could not be hydrogenated. One N atom per molecule was liberated by the Van Slyke procedure; the other N was nonbasic. $C_{13}H_{20\text{-}22}N_2O_6 \cdot \frac{1}{2}H_2O$: C = 50.31%; H = 6.88%; N = 9.17%. Sulfate: colorless crystals; solubilities the same as the base. Ultraviolet absorption spectrum maxima at 263 mμ (a = 26.6) in 0.1 *N* HCl; at 264 mμ (a = 25.3) in 0.1 *M* phosphate buffer at pH 7.0; and at 288 mμ (a = 40.6) in 0.1 N NaOH. Infrared spectrum given in reference 2. Most stable at pH 3.0; unstable at pH 7.0 and above. $[\alpha]_D^{26} = +54.5°$ (c = 1 per cent in water). Acetate: white needles, m.p. 263–266° C. Softens

at 130°C and resolidifies at 145°C. Soluble in water and soluble in lower alcohols and acetone while warm. Slightly soluble in ethyl acetate. $[\alpha]_D^{26} = +58°$ (c = 1 per cent in water). Acetylation of the acetate or free base with acetic anhydride gives a biologically inactive N-acetyl derivative: white needles; m.p. 254–255°C; $pK_a' = 8.4$ (2).

Biological activity: Moderately active on gram-positive and gram-negative bacteria. Resistance develops easily. No cross-resistance with other common antibiotics (1). Protects mice from infections with *Staph. aureus* and *K. pneumoniae* at high doses. No effect on infections with *D. pneumoniae, Pr. vulgaris, Sal. typhimurium,* or *M. tuberculosis.* Very slightly active against *Plasmodium lophurae* (chicks) but not *Schistosoma mansoni* (mice), helminths (rat), or protozoa (1). Protects X-ray irradiated rats against the transplantable human neoplasms H.S. No. 1 sarcoma and H. ep. No. 3 carcinoma when given 24 hours after tumor implantation. Ineffective against these tumors after they were established (4 days post-implantation) (4). Not effective against H. ep. No. 3 in mice, hamsters, or eggs (3). Active on sarcoma 180 (ascites), Ehrlich carcinoma (ascites), carcinoma 1025, glioma 26, Walter carcinosarcoma 256, and slightly active on adenocarcinoma EO771 (5). Active on a large number of transplanted mouse leukemias, including lines resistant to other antitumor agents (6).

Toxicity: LD_{50} (mice) 800 ± 27 mg per kg and 1550 ± 26 mg per kg (rat) intravenously (1).

References:

1. Pittillo, R. F. *et al.* Antibiotics Ann. 497–504, 1958–1959.
2. Haskell, T. H. and Bartz, Q. R. Antibiotics Ann. 505–509, 1958–1959.
3. Merker, P. C. and Woolley, G. W. Antibiotics Ann. 515–517, 1958–1959.
4. Teller, M. N. *et al.* Antibiotics Ann. 518–521, 1958–1959.
5. Sugiura, K. and Reilly, H. C. Antibiotics Ann. 522–527, 1958–1959.
6. Burchenal, J. H. *et al.* Antibiotics Ann. 528–532, 1958–1959.

Actinoflocin

Produced by: Streptomyces sp. resembling *S. albus* (3). This culture also produces six other antibiotics (3, 4).

Synonym: Kikuchi's third substance (1).

Method of extraction: I. Broth extracted with chloroform at acid pH. Chromatography of concentrated extract on cellulose powder. Washed with 20 per cent NH_4Cl and eluted with water (3, 4). II. Broth adsorbed on IRC-50 (H^+ phase) at pH 7.8 and eluted with 80 per cent acetone. Eluate extracted with chloroform at pH 5.0 (4).

Chemical and physical properties: Soluble in most organic solvents. Sparingly soluble in water. Ultraviolet absorption spectrum maxima at 231 and 276 mμ. Stable in solution from pH 2.0 to 8.0. Thermostable (2).

Biological activity: Active on *Streptococcus hemolyticus* at 1.56 μg per ml (2). Slightly active on *B. subtilis.* Not active on *Sarcina lutea, C. albicans* or *A. niger* (4). Slight activity on ascitic and solid forms of Ehrlich carcinoma and sarcoma 180 (2).

Toxicity: LD_{50} (mice) 3 mg per kg intraperitoneally (2).

References:

1. Kikuchi, K. J. Antibiotics (Japan) **8A:** 145–147, 1955.
2. Katagiri, K. *et al.* Chemotherapy (Tokyo) **4:** 143, 1956.
3. Sato, K. and Katagiri, K. Chemotherapy (Tokyo) **5:** 182–183, 1957.
4. Katagiri, K. *et al.* Shionogi Kenkyusho Nempo **7:** 715–723, 1957.

Actinoidin

Produced by: Nocardia (*Proactinomyces*) *actinoides.*

Method of extraction: Adsorption on activated carbon. Elution with 80 per cent aqueous acetone at pH 3.0. Purification by chromatography on "Permutit" (alumina-sodium silicate). Washed with distilled water and eluted with 5 per cent NaCl solution. Salt conversion: picrate to hydrochloride. Reprecipitation from methanol with acetone. Also forms a reineckate. Carboxylic cation exchange resins (H^+ form) can also be used to purify actinoidin.

Chemical and physical properties: Basic polypeptide. *HCl salt:* water-soluble, white amorphous powder. *Free base:* Little solubility in water except at pH 8.5. Slightly soluble in methanol and ethanol. Insoluble in butanol, acetone, ether, and other nonpolar solvents. Most stable at acid or neutral pH. N = 7 per cent (Kjeldahl); amino N = 2 per cent (Van Slyke). Positive Pauly, Molisch, and biuret reactions. Product of diazotization reacts with α-naphthol to give a brownish green color. Negative orcin, $FeCl_3$, Fehling, Seliwanoff tests, and negative test for amino sugars. Fehling reaction becomes positive after 3 to 5 minutes of heating in 5 per cent HCl. Mild acid hydrolysis yields two fractions: I = 5 per cent HCl-insoluble; II = 5 per cent HCl-soluble. Fraction I contains many

components giving positive Pauly reactions. Fraction II is a complex peptide.

Biological activity: Active on gram-positive but not gram-negative bacteria. No cross-resistance with penicillin, streptomycin, albomycin, erythromycin, or chlortetracycline. Resistance develops slowly. Active *in vivo* (mice) on pneumococcal infections; somewhat less active on staphylococcal or streptococcal infections. Activity considered equal to chlortetracycline and better than penicillin.

Toxicity: Mice tolerate 2 gm per kg intravenously. Not irritating when instilled as a 1 per cent solution into the eyes of rabbits. Strong inflammatory reaction at the site of a subcutaneous or intramuscular injection. Causes overgrowth of gram-negative bacteria in the intestine when given orally. Poorly absorbed from the intestinal tract.

Utilization: Limited by painful reactions at injection site.

Reference: 1. Shorin, V. A. *et al.* Antibiotiki **2(5):** 44–49, 1957.

Actinoleukin

Produced by: Streptomyces aureus and *Streptomyces* sp. (1, 3).

Synonym: Related to levomycin, antibiotic F43, and echinomycin.

Method of extraction: Broth-filtrate extracted with ethyl or butyl acetate. Extract decolorized with nitric acid-treated alumina. Effluent evaporated *in vacuo*. Can be purified by (a) chromatography on alumina with ethyl acetate or developer. Active fractions concentrated to dryness *in vacuo*, washed with petroleum ether, and crystallized from ethanol. (b) Countercurrent distribution (80 per cent aqueous methanol-benzene-carbon tetrachloride, 2:1:1); active fractions recrystallized from methanol-chloroform (7:3) (1, 3).

Chemical and physical properties: White platelets or needles; m.p. 213°C (decomposition). Soluble in chloroform and pyridine. Sparingly soluble in acetone, warm ethanol, methanol, ethyl and butyl acetates. Slightly soluble in carbon tetrachloride and benzene. Insoluble in water. Ultraviolet absorption spectrum maxima at 243 and 312 mμ (solvent not given). Infrared absorption spectrum in reference 3. $[\alpha]_D^{13.5} = -302°$ (c = 0.01 per cent in ethyl acetate). Negative $FeCl_3$, ninhydrin, Molisch, Sakaguchi, biuret, Tollen, and Fehling tests. Positive pine-splint test. Ehrlich test gives a yellow color. $C_{29-30}H_{40-42}N_6O_{7-8}S$: C = 56.41%; H = 6.74%; N = 13.06%; S = 5.01%. Molecular weight, 648 (Rast) (1, 3).

Biological activity: Active on gram-positive bacteria. Little or no activity against gram-negative bacteria (1). Kills HeLa cells at 0.06 μg per ml (2). Slight activity on Ehrlich ascites carcinoma (1, 3).

Toxicity: LD_{50} (mice) 1.5 mg per kg intraperitoneally (1).

References:

1. Ueda, M. *et al.* J. Antibiotics (Japan) **7A:** 125–126, 1954.
2. Umezawa, H. Giorn. microbiol. **2:** 160-193, 1956.
3. Ishihara, S. *et al.* J. Antibiotics (Japan) **11A:** 160–161, 1958.

Actinolysin

Produced by: Streptomyces albicans.

Method of extraction: Nutrient broth inoculated with a culture of the organism and incubated at 30° C for 4 to 5 days. The lysed culture is filtered.

Chemical and physical properties: Unstable, partly destroyed at 80°C and completely destroyed at 100°C. Can be separated into two constituents by means of dialysis; neither of these is active by itself. In contrast to actinophage, the agent is active upon dead cells as well as upon living.

Biological activity: Active only upon the culture from which it is isolated.

Utilization: Exerts a favorable effect in the treatment of actinomycotic infections.

Reference: 1. Dimitriev, S. and Suteev, G. Med. Parasitol. Parasitic Diseases (U. S. S. R.) **No. 4:** 1947.

Actinomycelin

Produced by: Streptomyces antibioticus.

Method of extraction: Broth-filtrate extracted with butanol. Extract dried *in vacuo*, washed with organic solvents, such as benzene, dissolved in ethanol, and filtered. Dried *in vacuo*. Purified by chromatography on alumina from butanol and developed with methanol and methyl acetate (3:1)

Chemical and physical properties: Neutral, yellowish green substance. Soluble in water at pH 7.0 and in ethanol (with intense fluorescence). Scarcely soluble in chloroform and amyl acetate; insoluble in ether and benzene. Treatment with concentrated HCl gives a red or gray color; precipitate forms on boiling. Positive Fehling and $FeCl_3$ tests. No reaction with alcoholic NaOH, picric acid, KCN, or sodium bisulfite. Decolorizes $KMnO_4$. More stable at pH 7.0 than at alkaline or acid pH. Thermolabile.

Biological activity: Active on gram-positive

bacteria. Not active on gram-negative bacteria, mycobacteria, or fungi.

Toxicity: 25 mg per kg lethal to rats.

Reference: 1. Cercos, A. P. Rev. invest. agr. **2:** 147–156, 1948.

Actinomycetin

Produced by: Streptomyces albus and *Streptomyces* sp. (1, 2).

Remarks: Antibacterial factor may be similar to mycomycin.

In discussing Feistmantel's (5) work on the close genetic relationship of the tuberculosis organism and the actinomycetes, based upon the reactions given to tuberculin and by preparations of *Nocardia farcinica* (the culture being designated as *Streptothrix farcinica* and the preparation as "farcin poison") in guinea pigs, Mathieson *et al.* (6) used in 1935 the term "actinomycetin" for the extract of the culture designated by them as *Actinomyces farcinica.* In 1937, Welsch (7) used the designation "actinomycetin" for the sterile filtrates of an actinomycete which possessed bacteriolytic properties.

Method of extraction: I. Broth-filtrate at pH 3.0 saturated to 75 per cent with ammonium sulfate. Precipitate taken up in water and reprecipitated by addition of alcohol or acetone at low temperatures. The lipoid antibacterial substance is extracted from the ammonium sulfate precipitate with ether. Ether concentrated to dryness. Residue taken up in ether, washed with 5 per cent HCl, then extracted into 5 per cent NaOH. Re-extracted into ether at acid pH, then into water at alkaline pH several times (2). The two peptidases (F_1 and F_2) of the staphylolytic principle (see below) can be separated by chromatography (3). II. Culture-filtrate concentrated *in vacuo* at 35°C while N_2 is bubbled through the solution. Concentrate treated with ammonium sulfate at 0°C and at pH 8.0. Precipitate which forms taken up in 0.033 *M* K_2HPO_4, centrifuged, and dialyzed against distilled water at low temperature (4).

Chemical and physical properties: Actinomycetin is a general term for all the products, having activity on microorganisms, which are produced by *S. albus* and other *Streptomyces* spp. It includes the following: (a) *A colilytic principle*, which is a protein and an enzyme and is not identical to the other proteases or enzymes present in the mixture (1). (b) *A staphylolytic principle* containing two peptidases, F_1 and F_2, which are weakly active alone, but synergistically active when combined. F_1: White powder or "pic unique" crystals; soluble in presence of electrolytes at >pH 5.0. Basic protein having no protease-like activity. F_2: Brownish powder, not completely purified; soluble in the presence of electrolytes at >pH 5.0. No protease-like activity (3). (c) *Lytic system active on living streptococci*, in part distinct from the staphylolytic principle. (d) *Activity on heat-killed or living pneumococci* represented by four lytic agents, at least two of which differ from the staphylolytic, colilytic, and streptolytic principles. (e) *Activity (lytic) on heat-killed but not living gram-negative rods*, occurring at 60–65°C more rapidly than thermal sterilization (1). (f) *Other enzymes* capable of digesting a variety of substrates, such as casein, keratin, fibrin, fibrinogen, and mucin (3). (g) *Antibacterial substance:* yellowish oily liquid. Acidic, probably unsaturated fatty acids. Soluble in dilute alkali, petroleum ether, ether, acetone, chloroform, and carbon tetrachloride. Less soluble in ethanol. Insoluble in water and dilute acids. Loses activity on exposure to air (2). (h) *Antiviral substance:* differs from the other agents present in the complex. Thermolabile (4).

Biological activity: The antibacterial substance (g) is active on certain gram-positive bacteria including *M. phlei.* Not active on gram-negative bacteria except *Flavobacterium* sp. Very active on certain protozoa, such as *Paramecium*, *Glaucoma*, and *Colpidium* sp. Biological activity inhibited by complex organic substances of a protein nature (2). The colilytic principle (a) is active on heat-killed *E. coli* (1). The staphylolytic principle (b) is active on the cell walls of living gram-positive bacteria (3). The antiviral substance (h) destroys the ability of red cells to be agglutinated by influenza MF 1 (A) and Lee (B), but not influenza PR 8 (A) or Newcastle disease virus; it destroys the hemagglutinin of influenza A (MF 1 and PR 8), influenza B (Lee), and influenza C, but not the Newcastle disease virus; it destroys the infectivity of influenza A (PR 8) in contact tests in eggs and mice. It is not active *in ovo.* It is active in protecting mice from influenza infections, if given by the intravenous route (4).

Toxicity: 1 gm given intraperitoneally kills mice in 18 hours or less (4). Antibacterial substance was toxic to tomato plant roots at 5 μg per ml (2).

Utilization: Study of the structure of bacterial cells (3).

References:

1. Welsch, M. *et al.* Proc. Intern. Congr. Biochem., 3rd Congr., Brussels, 1955, p. 413–415.
2. Welsch, M. Phénomènes d'antibiose chez les

actinomycetes. J. Duclot, Gembloux, France, 1957.
3. Salton, M. R. J. and Ghuysen, J. M. Biochim. et Biophys. Acta **24:** 160–173, 1957.
4. Malchair, R. Giorn. microbiol. **5:** 137–157, 1958.
5. Feistmantel, Dr. Centr. Bakteriol Parasitenk. Abt. 1, Orig. **36:** 282–290, 406–415, 1904.
6. Mathieson, D. R. *et al.* Am. J. Hyg. **21:** 405–431, 1935.
7. Welsch, M. Compt. rend. Soc. Biol. **122:** 244–246, 1937.

Actinomycins

Introduction: Actinomycin A, discovered by Waksman and Woodruff in 1940 (1), was relegated to a decade of oblivion because of its high toxicity (3). Following the fine work on actinomycin C (Brockmann and coworkers) and actinomycin B (by workers in England), and the report that actinomycin C was effective clinically against certain neoplastic diseases (21), interest in the actinomycins was vigorously renewed.

Altogether, actinomycins A, B, C, D, E, F, I, J, K, M, X, and Z, as well as others, have been reported. All are toxic, red substances that have similar biological properties. They can be extracted and purified in similar ways, but they differ in certain physical and chemical properties. The designations actinomycin A, actinomycin B, etc., do not refer to single substances but to complexes. The components of the actinomycin complexes are very closely related. Two different complexes may contain the same components, but in different proportions. One component may be found in one complex but not in another.

Brockmann and his coworkers refer to the various components by the letter of the complex, with a subordinate arabic number; *i.e.*, X_0, X_1, X_2, etc. As the numbers become larger, the Rf values become larger within a single complex, regardless of the solvent system used. Roussos and Vining (62), on the other hand, introduced another system, using subordinate Roman numerals. These numerals referred to a particular Rf value in a specific system of paper chromatography and applied to a component with that Rf, regardless of its complex. The numeral was used in conjunction with the letter of the complex to indicate the source of the component; *e.g.*, A_I, B_{II}, D_{IV}, etc.

Waksman *et al.* (77) proposed to clarify this confusion by terming all actinomycin complexes "mixtures." Each different component of these mixtures, when purified and characterized, would be given a roman numeral, with no reference being made to the complex which was the source of the component.

A_I, B_I, $X_{0\beta}$ = Actinomycin I
A_{II}, B_{II} = Actinomycin II
A_{III}, B_{III} = Actinomycin III
A_{IV}, B_{IV}, D_{IV}, C_1, I_1, X_1 = Actinomycin IV
A_V, B_V, X_2 = Actinomycin V
C_2 = Actinomycin VI
C_3 = Actinomycin VII

Thus, what has been called the actinomycin A complex becomes a mixture containing actinomycins I to V. All others, such as the new biosynthetic actinomycins (*e.g.*, E, F) would become "mixtures" until their components were more fully characterized. This new system will be used insofar as possible in the following discussion, although previously used terminology will be employed parenthetically for historical clarity.

It is evident from this, as previously mentioned, that the same components can appear in different complexes. Roussos and Vining (62) found by circular paper chromatography that four "actinomycin mixtures" (A, B, D, and X) contained actinomycins I, II, III, IV, and V, but in different proportions (Table 38). This was further verified when actinomycins I, IV, and V isolated from the different mixtures were shown to have almost completely identical physical and chemical properties.

In general, actinomycins have a m.p. of about 215–255°C. They are soluble in benzene, ethanol, and acetone, slightly soluble in water and ether; insoluble in aqueous dilute alkali and petroleum ether. All have peak absorption at about 440 to 450 mμ and about 240 mμ in ultraviolet light; many also show a peak at about 415 to 430 mμ. All reported thus far are levorotatory. Actinomycins give a transient purple color in ethanolic NaOH, and a dark red color in concentrated HCl. They are most stable at pH 6 to 7, relatively stable at acid pH, and destroyed at alkaline pH.

Actinomycins are polypeptides linked to a chromophoric quinoid moiety. This chromophore has been shown to be 3-amino-1,8-dimethylphenoxazone-(2)-dicarboxylic acid (4, 5) for actinomycins I ($X_{0\beta}$), IV(D_{IV}, C_I, I_I), V(X_2, B_V), VI(C_2), and VII(C_3), and is believed to be the same for all actinomycins (see formula in Chapter 6 and references 56, 57, 65, 98). This belief is based on the fact that the main barium hydroxide hydrolysis degradation product of these components is the same (see references 25, 39, 45, 47, 65). The structure of despeptido actinomycin is shown here:

TABLE 38

Percentage of actinomycins contained in various mixtures (62, 77)[a]

Mixture	Actinomycin						
	I	II	III	IV	V	VI[b]	VII
A type	6.6	2.9	tr.[c]	66.7	23.8		
B type	9.6	tr.	tr.	28.1	59.3		
C[d] type				18.1		45.7	34.4
D type	tr.	tr.	tr.	100.0	tr.		
X[d] type	5.1[e]			5.1	88.6		
I[f]				86.5			

[a] See references to "Actinomycins," Part A.

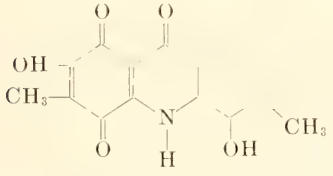
[b] Roussos and Vining (62) described a VI component in the "B type" mixture, but this is not synonymous with the VI of Waksman *et al.* (77).

[c] tr. = trace.

[d] Contains other minor components not listed in the table.

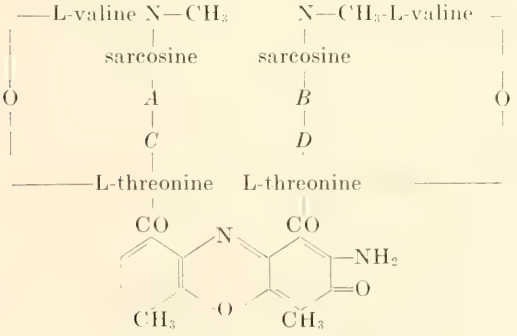
[e] Probably represents $X_{0\beta} + X_{0\alpha}$ + another less well defined fraction.

[f] Contains other major and minor components not listed in the table.



The polypeptide portions of the various actinomycins contain a variety of amino acids differing in their identity, arrangement, and number. Physical and chemical variations observed between various components appear to be caused by these differences. To date, all actinomycins described contain L-threonine and sarcosine (Table 39).

The complete structures of actinomycins I to VII are shown below, and some of their properties are given in Table 40 (44, 57, 63, 65, 95, 101).



Basic structure of actinomycins

Variations from one actinomycin to the other occur at the locations marked *A*, *B*, *C*, and *D* as follows:

Actinomycin I: *A* = L-proline, *B* = hydroxyproline, *C* = *D* = D-valine (44).

Actinomycin II: *A* = *B* = sarcosine, *C* = *D* = D-valine (101).

Actinomycin III: *A* = proline, *B* = sarcosine, *C* = *D* = D-valine (101).

Actinomycin IV: *A* = *B* = L-proline, *C* = *D* = D-valine (65).

Actinomycin V: *A* = proline, *B* = 4-ketoproline, *C* = *D* = D-valine (95).

Actinomycin VI: *C* = D-valine, *D* = D-alloisoleucine, *A* = *B* = L-proline (57).

Actinomycin VII: *C* = *D* = D-alloisoleucine, *A* = *B* = L-proline (57).

Actinomycins which have been characterized biologically are active on gram-positive bacteria, less active on mycobacteria, and inactive on fungi and gram-negative bacteria, although their activity varies quantitatively. One report of antiviral activity exists (80). Many are active on tumors in animals, and certain actinomycins have been reported to have a temporary activity on neoplasms in man (21, 27, 41–43, 49, 76). All are very toxic substances. Many cause splenic atrophy in animals after multiple doses (60).

Actinomycin-producers thus far reported are all members of the family *Streptomycetaceae*. Some actinomycin-producing cultures have been found under various conditions to form the components of their "mixtures" in proportions differing from what was considered the norm, or even to yield new actinomycins altogether. A certain

TABLE 39

Amino acid composition of various actinomycins

Data given as moles of amino acid per mole of actinomycin (molecular weight, 1200).

Amino acids	Actinomycins																				
	I	II	III	IV	V	VI	VII	E_1	E_2	F_0	F_1	F_2	F_3	F_4	F_5	I_0	Z_0	Z_1	Z_5	1048A I, II, III	2104L I	2104L II
L-Threonine	2	2	2	2	2	2	2	+[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Valine	2	2	2	2	2	1	0	0	0	+	+	+	0	0	+	+	+	+	+	+	0	+
L-Proline	1	0	1	2	1	2	2	+	+	+	0	+	0	+	+	+	0	0	0	+	+	+
Sarcosine	2	4	3	2	2	2	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N-CH_3-L-valine	2	2	2	2	2	2	2	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Alloisoleucine	0	0	0	0	0	1	2	+	+	0	+	+	+	+	+	0	0	0	0	0	+	+
4-Hydroxyproline	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4-Ketoproline	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Methylisoleucine	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Methylalanine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	0

[a] Designates presence of amino acid.

Streptomyces sp. was shown to produce, in early stages of growth, an actinomycin similar in the proportions of its components to the "B mixture," whereas at later stages of growth, the proportion of components was changed to that of an "A mixture." This was demonstrated by circular paper chromatography in 5 per cent Na-o-cresotinate and ethyl acetate-n-butyl ether (2:1). Under different nutritional conditions, this culture could produce only A-, or only B-type "mixtures" (61, 66). A "C-mixture"-producing culture formed VII (C_3) as the major component actinomycin in early growth stages, whereas later, VI (C_2) became the major component (28). Addition of sarcosine to an L-glutamic acid-containing synthetic medium in which an "A mixture"-producer was grown changed the proportions of the actinomycins normally formed. Amounts of actinomycins IV and V were decreased, and II and III increased (100).

It has also been possible to produce new actinomycins when different amino acids or amino acid derivatives (such as oligopeptides) were added to the culture media in which "C" or "A mixture"-producers were grown (58, 86). These new actinomycins are referred to below as "biosynthetic" actinomycin mixtures.

In the descriptions which follow, no data have been included from reports in which the complex was tested as though it were a single entity instead of a mixture; only references to such publications will be given. The heterogeneity of the complexes makes such data invalid.

Actinomycin Mixtures (A, B, D, J, X) Containing Actinomycins I, II, III, IV, and V

Produced by: Streptomyces antibioticus (2, 82) (A, B mixtures); *S. parvus* and others (8, 31, 35, 80) (A mixture); *S. kitasawaensis* (103) (A mixture); *Streptomyces* sp. (10, 13) (B mixture); *S. parvullus* (31) (D mixture); *S. flavus* (6, 17) (J mixture); *S. flaveolus* (17) (J mixture); *S. griseus* (*S. parvus*) (71) (X mixture); *S. michiganensis* (72, 99) (X mixture); *S. galbus* (99) (X mixture); *S galbus* var. *achromogenes* (99) (X mixture); *S. murinus* (99) (X mixture); *S. lanatus* (99) (X mixture); and *Streptomyces* sp. (18, 22, 23, 28).

Synonyms: Antibiotic X 45 (B mixture) (13). Actinoflavin (J mixture) (9).

Method of extraction: I. Broth and/or mycelium extracted with ether, n-butanol, benzene, or butyl acetate. Extract concentrated to dryness. Residue treated with petroleum ether and extracted into acetone, benzene, or cold chloroform, filtered and evaporated to dryness. Recrystallized from cold ethanol on addition of successive aliquots of petroleum ether, or from acetone, ether, warm ethanol, or other solvents. Purified by chromatography on alumina from benzene and elution with 30 per cent acetone in benzene or with ethyl acetate (4, 10, 23, 32). Actinomycins I, II, III, IV, and V are partially separated by chromatography of the crude mixture on acid-washed alumina from benzene-chloroform (3:1) and elution with the same mixture, gradually changing the proportion to 1:3. Particular fractions are further separated by rechromatographing as before, but gradually

TABLE 40

*Chemical and physical properties of actinomycins I to VII**

Actinomycins	Previous designations	Crystal form	Melting point†	Specific rotation	λ_{max}			E‡			Analysis
					In methanol or ethanol (95%)	In $CHCl_3$-ethanol (1:10)		443 mμ (methanol)	444 mμ (95% ethanol)	446 mμ (cyclohexane)	
			°C		mμ	mμ	(log ε =)				%
I	A_1, B_1, $X_{0\beta}$	Plates or needles	237–238 or 245–247	$[\alpha]_D^{23} = -235°$ (c = 0.25% in ethanol)	242.0–242.5 441			17.1	17.8–18.0		C = 58.06 H = 6.93 N = 12.73
II	A_{II}, B_{II}	Plates	215–216	$[\alpha]_D^{19} = -157°$ (c = 0.24% in $CHCl_3$)	237 447	237 429 447	(4.55) (4.33) (4.37)				C = 58.0 H = 6.83 N = 13.8
III	A_{III}, B_{III}	Prisms	237–238	$[\alpha]_D^{19} = -205°$ (c = 0.22% in $CHCl_3$)	240 450	240 430 450	(4.43) (4.38) (4.41)				C = 58.4 H = 7.2 N = 13.1
IV	A_{IV}, B_{IV}, D_{IV}, C_1, I_1, X_1	Prisms, hexagonal bipyramids, or needles	235.5–236.5 or 240–243	$[\alpha]_D^{23} = -261$ to $268°$ (c = 0.25% in 95% ethanol) or $[\alpha]_D^{20} = -349° (\pm 10°)$ (c = 0.26% in methanol)	242.0 443.0–444.0			20.5	18.8–19.6	20.7	
V	A_V, B_V, X_2	Fine needles or rhombic plates	245–246.5	$[\alpha]_D^{23} = -320$ to $323°$ (c = 0.25% in 95% ethanol)	240.0 445.0			18.6	19.2–19.6		
VI	C_2	Same as IV	237–239	$[\alpha]_D^{19} = -325° (\pm 10°)$ (c = 0.24% in methanol)	*ca.* 238 415 443			19.9			C = 58.80 H = 6.90 N = 12.76
VII	C_3	Same as IV	232–235	$[\alpha]_D^{19} = -328° (\pm 10°)$ (c = 0.24% in methanol)	*ca.* 238 415 443			18.8			

* Data from "Actinomycins," references 25, 32, 36, 37, 44, 59, 62, and 101.

† Kofler hot stage.

‡ Absorption of a 1 cm thickness of a 1 gm/liter solution.

proceeding to a 2:3 ratio in the eluent. Complete separation is made by preparative paper chromatography (N-dibutyl ether-tetrachloroethane-10 per cent sodium o-cresotinate) (62, 101).

Chemical and physical properties: These mixtures contain actinomycins I(A_I, B_I, $X_{0\beta}$), II(A_{II}, B_{II}), III(A_{III}, B_{III}), IV(A_{IV}, B_{IV}, D_{IV}, X_1), and V (A_V, B_V, X_2) in varying proportions (See Table 38). "J mixture" has been identified with "X mixture" (83), but precise data have not been published. The A, B, and X mixtures also contain a number of other actinomycins, not yet characterized, in trace amounts. Physical and chemical properties of the major actinomycins I to V are given in Table 40. The infrared spectrum of IV is given in reference 36, and infrared data on II and III are given in reference 101. Amino acid content of the peptide moieties of actinomycins I to V is given in Table 39 (24, 77). For information on the various mixtures see references 1, 4 (A mixture); 13, 19, 62, 82 (B); 32 (D); 6, 9, 12, 17 (J); 25, 28, 37, 44, 46, 62 (X).

Biological activity: Actinomycins I to V are active on gram-positive bacteria, less active on mycobacteria, and inactive on fungi (102). Some typical activities of components I, IV, and V are given in Table 41. Actinomycins II and III were about half as active as IV *in vitro* (86). Tested in mice against Gardner lymphosarcoma (6C3HED) (ascitic form), actinomycins I and V had much less activity than IV, and II and III had equal or better activity than IV (60, 86). Actinomycin IV has been reported active on the following tumors: Crocker sarcoma 180 (50), malignant melanoma S91, mammary adenocarcinoma, myeloid and lymphoid leukemia (53), Ehrlich carcinoma (ascitic form), Krebs 2 ascitic carcinoma (54), Ridgeway osteogenic sarcoma, carcinoma 1025 (90), ME 1 melanoma (human amelanotic), MFS1 myxofibrosarcoma, KB (Eagle human carcinoma), P1534 mouse lymphatic leukemia (88), RC mammary carcinoma (91), mammary adenocarcinomas C 755 and C 3HBA, thymoma (solid), and the ascites form of an ovarian carcinoma (70). Information on the various mixtures is given in references 26 and 55. Treatment of actinomycins with methanolic sodium hydroxide opens the lactone rings in the polypeptide chains. The resulting compounds are called actinomycinic acids. The actinomycinic acid of actinomycin S_3 has no antibacterial activity but some antitumor activity (108).

Toxicity: LD_{50} (mice) subcutaneously (60): actinomycin I, > 8 mg per kg; actinomycin IV, 1.1 mg per kg; actinomycin V, 0.35 mg per kg. LD_{50} (mice) intraperitoneally (86): actinomycin II, 6 mg per kg; actinomycin III, 1.5 mg per kg. Actinomycin I had much less toxicity than IV and V, as measured by splenic atrophy (60). Actinomycin IV (D) had a teratogenic effect on the rat foetus at 10 μg per mother rat (4). It was also intensely irritating to skin and subcutaneous tissue. In human beings, side effects included depletion of bone marrow, stomatitis, pigmentation of the skin, and gastrointestinal disturbances (93). Other toxicity studies with actinomycin IV in animals (70) and normal and neoplastic tissue cell cultures (68) have been reported.

TABLE 41

Biological activity of actinomycin components I, IV, and V against four test organisms (60)

Actinomycin	Minimal inhibitory concentration			
	Staph. aureus	*B. subtilis*	*B. cereus*	*Sarcina lutea*
	μg/ml			
I	0.4	0.48	0.5	0.175
IV	0.14	0.068	0.07	0.066
V	0.07	0.042	0.052	0.035

Utilization: Used experimentally in the treatment of certain neoplastic diseases (69, 92, 93).

Actinomycin mixtures (C and AA-AC) Containing Actinomycins IV, VI, and VII

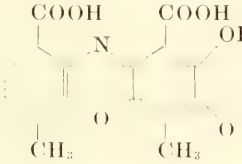
Produced by: Streptomyces chrysomallus (20), *S. griseus* (84), *S. chrysomallus* var. *fumigatus* (99), and *Streptomyces* sp. (5, 23, 28, 64, 85, 87).

Synonym: Antibiotic HBF 386 ("C mixture").

Method of extraction: Ground dried mycelium extracted with benzene. Broth extracted with butyl acetate. Broth-extract concentrated *in vacuo*, residue dissolved in benzene-extract from mycelium, and the whole chromatographed on alumina. Eluted with ethyl acetate; eluate concentrated. Addition of carbon disulfide to concentrate gives precipitate of crude "C mixture." Washed with ethyl acetate-carbon disulfide (1:2) and carbon disulfide. Recrystallized from ethyl acetate, ether, methanol, or ethanol. Separation of major components by countercurrent distribution (5 per cent Na *p*-xylol sulfonate-methyl butyl ether-n-dibutyl ether) (16, 23) or by chromatography on alumina (33).

Chemical and physical properties: "*C mixture*" contains three major components, actinomycins IV(C_1), VI(C_2), and VII(C_3), and a number of

trace components including C_0, C_{0a}, C_{1a}, C_{2a}, C_{3a}, and C_4 (36, 37, 87). These components have the following R_{C_2} values (Rf value relative to actinomycin VI(C_2)) (87): $C_0 = 0.2$; VI(C_2) = 1.0; $C_{3a} = 1.56$; IV(C_1) = 0.69; $C_{2a} = 1.15$; $C_4 = 1.7$; $C_{1a} = 0.8$; VII (C_3) = 1.39. *System:* n-Dibutyl ether, n-butanol, and 5 per cent aqueous β-naphthalene sulfonic acid. Other components have been reported formed when a "C mixture"-producing streptomycete was grown in the presence of various amino acids (64) (see actinomycin mixtures E and F). The infrared spectrum of actinomycin IV (C_1) is given in reference 36 and that of actinomycin VII (C_3) in reference 37. The other physical and chemical properties of actinomycins IV (C_1), VI(C_2), and VII(C_3) are given in Table 40. Their amino acid content is given in Table 39 (24, 77). On mild acid hydrolysis, actinomycin VII(C_3) splits off one molecule of ammonia and forms a "desamino actinomycin" ($C_{64}H_{89}O_{17}N_{11}$; brick-red rhomboid crystals; m.p. 239°C). The chromophore of this degradation product (3 hydroxy-1,8 dimethylphenoxazone-(2)-dicarboxylic acid-(4,5) (40) is shown below:



"*AA-AC mixture*", contains two actinomycins in major amounts, IV (AA) and VII(AC), and a minor component with an amino acid content like that of VII, but not as yet completely characterized (30, 33, 83). References giving data concerning the "mixtures" include 16, 29, 36, 37, 40 ("C mixture"); and 14, 30, and 33 ("AA-AC mixture").

Biological activity: The minimal inhibitory concentrations of some of the component actinomycins of the "C mixture" against *B. subtilis* are as follows (in μg per ml): actinomycin IV (C_1), 0.2; VI (C_2), 0.14; VII (C_3), 0.2; C_0, 19; $C_0\alpha$, 50; and $C_0\beta$, 28. The activity against mammary carcinoma TM 8013 and lymphosarcoma T 24 179 of the "C mixture" is accounted for by actinomycins IV (C_1), VI(C_2), and VII(C_3); VII (C_3) being the least active (67). "Desaminoactinomycin" has less than $\frac{1}{100}$ the activity of the parent compound on *Staph. aureus* (40). References giving data on the biological activity of the "C mixture" include 11, 27, 34, 89, and 91; for the "AA-AC mixture," reference 5.

Toxicity: LD_{50} (mice, no route given, mg per kg): IV (C_1), 1.8; VI (C_2), 0.9; VII (C_3), 1.6; C_0, 8; $C_0\alpha$, 24; and $C_0\beta$, 41 (67). References giving data on the toxicity of the "mixtures" include 5 and 27.

Utilization: Same as previous "mixtures." See references 41–43 and 49. Some anti-allergy activity has been reported (42).

Actinomycin B or J_2

A preparation designated as actinomycin J_2 is a mixture of actinomycin J_1 and duodecyl ester of 5-ketostearic acid. The last compound has no antibiotic activity. Actinomycin J_2 is very probably the equivalent of Waksman and Woodruff's actinomycin B. Later Waksman, Geiger, and Reynolds proposed dropping the term actinomycin B to designate this fraction, since obviously it is nothing else than a mixture of actinomycin A with inert material. They proposed calling actinomycin A, *actinomycin* (1, 105, 106).

Actinomycin Mixture "I"

Produced by: Streptomyces parvullus (71, 72), *S. antibioticus* (99), and *Streptomyces* sp. (38).

Synonym: This mixture may contain the same actinomycins as the "C mixture," but in different proportions (77).

Method of extraction: No data. Probably the same as for the other "mixtures" described.

Chemical and physical properties: Contains one major component, actinomycin IV (I_1), and four less well described components, I_0, I_{0a}, I_2, and I_3 (37, 77). Chemical and physical data on actinomycin IV(I_1) are given in Table 40. The amino acid content of IV (I_1) and I_0 is given in Table 39. I_0: Hexagonal bipyramids of needles; m.p. 242 to 243°C. Specific extinction at 446 mμ = 20.5 (cyclohexane) (37).

The actinomycins described below have been less well characterized. Whether they contain any of the known actinomycins (I to VII) is a matter for further research. They fall into three categories: Category 1: Actinomycins which are known to be "mixtures" for which a certain amount of data on the component actinomycins is available. Category 2: "Mixtures" for which no data on the component actinomycins are available. Only references to the original papers will be given. Category 3: "Actinomycins" which may be composed of one, or more probably, of a mixture of actinomycins. All important data have been included here, even though subsequent work may show the substance to be a "mixture." In all cases, the original author's terminology has been used.

CATEGORY 1:

Mixtures of actinomycins for which some component data have been published.

Biosynthetic Actinomycin Mixtures E and F

Produced by: Streptomyces sp.

Remarks: This organism, which normally produced an actinomycin mixture of the "C type," produced another mixture containing entirely new actinomycins ("E type") when DL-isoleucine was added to the medium. It produced still another mixture ("F type"), along with actinomycins VI(C_2) and VII(C_3) when sarcosine was added (58, 59).

Method of extraction: Actinomycins of the "F mixture" were separated by chromatography on alumina (59).

Chemical and physical properties: Mixture "E" contains two major component actinomycins, E_1 and E_2. E_1: C = 58.89%; H = 7.21%; N = 12.28%. Specific extinction at 444 mμ is 18.2 (ethanol). E_2: C = 52.21%; H = 7.20%; N = 11.96%. Specific extinction at 444 mμ is 19.0 (ethanol). Infrared spectra of E_1 and E_2 are given in reference 59. Amino acid content is given in Table 39. The n-methyl isoleucine probably replaces the N-methyl valine of actinomycin VII (C_3).

"Mixture F" contains six component actinomycins, F_0 to F_5. All have the same ultraviolet and infrared spectra. Specific extinctions at 444 mμ (ethanol): F_1 = 19.0; F_2 = 19.4; F_3 = 18.5; F_4 = 19.0; F_5 = 17.8 (59). See Table 39 for the amino acid content of the F actinomycins.

Biological activity: E_1 has 90 per cent of the activity of actinomycin VI (C_2) against *B. subtilis*; E_2, 82 per cent; F_0, 74 per cent; F_1, 59 per cent; F_2, 71 per cent; F_3, 47 per cent; F_4, 55 per cent; and F_5, 72 per cent (59). Against Walker carcinoma (rat), E_1 had slight activity; E_2, none; F_0 was active at 5 × 60 μg per kg; F_1, F_2, F_3, and F_4 were active at high repeated doses (200 to 1000 μg per kg); F_5 was active at 100 μg per kg (single dose). Against Jensen sarcoma (rats), F_1 (the only one tested) was active. With Yoshida sarcoma (rats) as the test tumor, F_0, F_1, F_2, and F_4 were inactive at 6 × 60 μg per kg (highest level tested). Against Rous sarcoma (chickens), E_1, E_2, F_1, F_4, and F_5 were active (75).

Toxicity: E_1, E_2, and F_5 were as toxic as actinomycin IV (D); F_1 was 1/8 to 1/10 as toxic as IV; F_2, 1/2 as toxic; F_3 and F_4, 1/5 as toxic (75).

Utilization: See reference 75.

Actinomycin Mixture "K"

Produced by: Streptomyces melanochromogenes (73).

Method of extraction: Extracted from broth with benzene, and from mycelium with acetone-benzene. Purified by chromatography on alumina (73).

Chemical and physical properties: Contains three components. Acid hydrolysates of the "mixture" contain threonine, sarcosine, proline, valine, and isoleucine (73).

Biological activity: Typical of the actinomycins (73).

Toxicity: No valid data (73).

Actinomycin Mixture "M"

Produced by: Streptomyces sp. Has an antimitotic effect (62a).

Synonym: "Giolitti actinomycin" (63). Probably a mixture of the "B" or "X" type (48, 83).

Method of extraction: Similar to that of the other actinomycins (63).

Chemical and physical properties: Has three components with Rf values of 0.2 (trace), 1.0, and 1.86, and a fourth variably present (Rf = 2.6) as demonstrated by circular paper chromatography (ethyl acetate-n-butyl ether-2 per cent aqueous naphthalene-2-sulfonic acid, 1:1:2) (63).

Biological activity: Same as the other actinomycins (63). For data on the mixture see reference 74.

Toxicity: No valid data (63).

Actinomycin P_2

Synonym: Antibiotic PA 126-P_2.

Toxicity: Said to be less toxic to animals than actinomycins C and D (107).

Actinomycin Mixture "Z"

Produced by: Streptomyces fradiae (83).

Method of extraction: Culture-filtrate extracted with ethyl acetate. Extract concentrated, then evaporated *in vacuo* to an oily residue. Addition of petroleum ether precipitates Z. Chromatographed on aluminum oxide from benzene and developed with benzene-absolute CCl_4 (65:70), then chloroform-methanol (49:1), followed by the same mixture (19:1). Active fractions concentrated *in vacuo*. Residue taken up in acetone. Addition of ether gives "Z mixture." Components separated by chromatography on alumina with benzene as solvent. Developed with chloroform-methanol (99:1) to give Z_2, Z_3, Z_4, and Z_5. Z_1 follows further elution with same developer, Z_0 is eluted with $CHCl_3$-methanol (97:3). Z_0-and Z_1-containing fractions are each rechromatographed on alumina. Z_1 crystallized from acetone-ether. The mixure of Z_2, Z_3, and Z_4 is never separated. Z_5 is also crystallized from acetone-ether (83).

Chemical and physical properties: Contains six components, three of which were not separated. Amino acid content of Z_0, Z_1, and Z_5 is shown in Table 39. Z_0: Amorphous orange-brown powder. Decomposes with darkening at about 250°C. Ultraviolet absorption spectrum maxima at 236 and 437 mμ (log $E^{1\%}_{1cm}$ = 2.17 or 2.44). Z_1: Orange-red crystals; m.p. 256–260°C (decomposition); $[\alpha]_D$ = −362° (c = 0.185 per cent in $CHCl_3$). Ultraviolet absorption spectrum maxima at 240 mμ (log $E^{1\%}_{1cm}$ = 2.25), 427 mμ ($E^{1\%}_{1cm}$ 2.00), and 442 mμ (log $E^{1\%}_{1cm}$ 2.01). Infrared spectrum identical to actinomycin "X mixture." C = 53.97%; H = 6.97%; N = 12.3%; N-CH_3 = 7.48%. Z_5: Short red rods, in clusters; m.p. 261–267°C (decomposition). $[\alpha]_D$ = −284° (c = 0.244 per cent in $CHCl_3$). Ultraviolet absorption maxima at 240 mμ (log $E^{1\%}_{1cm}$ 2.40), 428 mμ (log $E^{1\%}_{1cm}$ = 2.21), and 443 mμ (log $E^{1\%}_{1cm}$ = 2.24). C = 55.71% H = 6.44%; and N = 12.25% (83).

Actinomycin Mixture 1048a

Produced by: Streptomyces sp. (78).

Method of extraction: Acetone-extract of mycelium evaporated under reduced pressure, and residue taken up in ethyl acetate; precipitated on addition of petroleum ether. Recrystallized from ethyl acetate. Purified by countercurrent distribution (aqueous 1.7 per cent Na β-naphthalene-sulfonate and methyl butyl ether), and chromatography on alumina with acetone as eluent (78).

Chemical and physical properties: Contains three major components, I, II, and III, and a minor component, IV (author's designations). See Table 39 for the amino acid content of I, II, and III. *I*: m.p. 240–244°C (decomposition). Ultraviolet absorption spectrum maxima at 238 mμ ($E^{1\%}_{1cm}$ 243) and 440 mμ ($E^{1\%}_{1cm}$ 160). *II*: m.p. 238–240°C (decomposition). Ultraviolet absorption spectrum maxima at 240 mμ ($E^{1\%}_{1cm}$ 281) and 444 mμ ($E^{1\%}_{1cm}$ 191). *III*: m.p. 240–242°C (decomposition); ultraviolet absorption spectrum maxima at 240 mμ ($E^{1\%}_{1cm}$ 308) and 440 mμ ($E^{1\%}_{1cm}$ 209). *IV*: m.p. 239–243°C (decomposition); ultraviolet absorption spectrum maxima at 240 ($E^{1\%}_{1cm}$ 233) and 442 ($E^{1\%}_{1cm}$ 162) (78).

Biological activity: Order of activity against *Staph. aureus* and *B. subtilis:* I = II > III > IV. In antitumor activity (Ehrlich ascites carcinoma), all are equal (78).

Toxicity: I and II: LD_{50} (mice) 0.49 mg per kg (no route given). *III*: LD_{50} (mice) 0.89 mg per kg (no route given) (78).

Actinomycin Mixture 2104L

Produced by: Streptomyces sp. (79).

Method of extraction: See actinomycin 1048A.

Chemical and physical properties: Contains two major components, I and II, and two minor ones, III and IV (author's designations, not related to the usual I, II, III, and IV). *I*: m.p. 237–240°C. Ultraviolet absorption spectrum maxima at 240 mμ ($E^{1\%}_{1cm}$ 296) and 444 mμ ($E^{1\%}_{1cm}$ 203). $[\alpha]^{16}_D$ = −349.2°. Contains threonine, sarcosine, proline, n-methyl valine, and alloisoleucine. *II*: m.p. 230–232°C. Ultraviolet absorption spectrum maxima at 240 mμ ($E^{1\%}_{1cm}$ 270) and 440 mμ ($E^{1\%}_{1cm}$ 180). $[\alpha]^{16}_D$ = −284.0°. Contains threonine, sarcosine, proline, valine, N-methyl valine, and alloisoleucine (79).

Biological activity: Typical of the actinomycins (79).

Toxicity: Mice tolerate at least 50 μg per kg for 5 days, intraperitoneally (79).

CATEGORY 2:

Substances known to be "mixtures," but for which no component data is available

(1) Actinomycin 4A-2 (51).

(2) Actinomcyin Mixture — ? (96, 97).

CATEGORY 3:

It is not known whether these substances are single actinomycins or "mixtures"

Actinomycin 1

Produced by: Micromonospora sp. resembling *M. globosa* (15).

Method of extraction: Broth extracted with ether, and extract concentrated. Addition of petroleum to concentrate gives amorphous powder. Crystallization from aqueous ethanol and recrystallization from same solvent or a benzene-ether mixture (15).

Chemical and physical properties: m.p. 251–252°C. Ultraviolet absorption maxima at 239 mμ ($E^{1\%}_{1cm}$ 272) and 440 mμ ($E^{1\%}_{1cm}$ 192). Acid hydrolysis products include threonine, proline, valine, N-methyl valine, and sarcosine. C = 60.06%; H = 6.99%; N = 12.62% (15).

Biological activity: Active on gram-positive organisms, mycobacteria, but not *Sacch. cerevisiae* or *A. niger* at 50 μg per ml (15).

Actinomycin 2

Produced by: Streptomyces sp. (7).

Method of extraction: Same as for A-type mixture (7).

Chemical and physical properties: Red crystals, m.p. 248–250°C (decomposition). Soluble in diethyl ether; insoluble in petroleum ether. Ultraviolet absorption maxima at 446 mμ ($E^{1\%}_{1cm}$ 160 to 170), and 236 or 242 mμ ($E^{1\%}_{1cm}$ 265 or 230). $[\alpha]^{25}_{D} = -260$ to $-268°$ (7).

Biological activity: Typical of the actinomycins (7).

Toxicity: 10 μg per 20 gm mouse is lethal, intravenously (7).

Actinomycin 3

Produced by: Streptomyces chrysomallus (81).

Method of extraction: Present in culture-broth and mycelium (81).

Chemical and physical properties: Red substance; m.p. 254°C (decomposition). Soluble in acetone and chloroform; moderately soluble in benzene, ethanol, and ethyl acetate; less soluble in ether; very sparingly soluble in carbon tetrachloride and water; insoluble in petroleum ether. Yellow-green fluorescence in ultraviolet light. Ultraviolet absorption spectrum maximum at 450 mμ (acetone and alcohol). Contains six amino acids, including L-valine, L-proline, and L-threonine. Forms a biologically active, said to be less toxic than other actinomycins, water-soluble degradation product (81).

Biological activity: Typical of actinomycins (81).

Toxicity: 3.3 mg per kg is toxic to mice, intravenously or intraperitoneally (81).

Actinomycin 4

Produced by: Streptomyces sp. This culture also produces a eurocidin-like substance (52).

Method of extraction: Essentially similar to that for the C-type "mixture" (52).

Chemical and physical properties: Red hexagonal plates; m.p. 252–254°C. Ultraviolet absorption maxima at about 242 mμ ($E^{1\%}_{1cm}$ 40) and 440 to 450 mμ. Infrared spectrum given in reference 1; said to be similar to that of actinomycin C. C = 57.23%; H = 6.32%; N = 12.55% (52).

Biological activity: Typical of the actinomycins. Active on Ehrlich (ascites) carcinoma in mice (52).

Toxicity: 150 μg per kg is lethal to mice in 7 days, but 75 μg per kg is tolerated (52).

Aurantin

Produced by: A *Streptomyces* of the *aurantiacus* group (104).

Synonym: Aurantin is an actinomycin similar to actinomycin C.

Chemical and physical properties: Dark red hexagonal bipyramidal crystals; m.p. 251–253°C.

Biological activity: Active against gram-positive bacteria. Streptococci and *B. mycoides* completely inhibited by 0.04 μg per ml; staphylococci and *B. subtilis* by 0.4 μg per ml. Active in mice against Ehrlich carcinoma, sarcoma 180, and lymphoma L 10. Active in rats against sarcoma M 1, sarcoma 4 J, and Guérin carcinoma.

Toxicity: A single injection of 900 to 1000 μg per kg intraperitoneally kills more than 50 per cent of the mice. A single dose of 500 μg per kg is tolerated by the animals. Rats are more easily killed than mice by aurantin.

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Actinone

Produced by: Streptomyces sp. resembling *S. antibioticus*.

Method of extraction: Broth-filtrate stirred with activated carbon. Elution with 95 per cent methanol. Eluate concentrated *in vacuo*. Acetone added to precipitate impurities. Filtrate freeze-dried. Purified by precipitating impurities from an absolute methanol solution with cold ether, evaporation *in vacuo*, and extraction with ether. Ether-insoluble fraction is lyophilized to give actinone.

Chemical and physical properties: Contains <1 per cent nitrogen. Insoluble in chloroform. Soluble in ether, butyl acetate, benzene, and petroleum ether.

Biological activity: Active against *Saccharomyces*, *Willia*, and *Trichophyton* species. Not active on bacteria or other fungi.

Toxicity: Mice tolerate 1 gm per kg intravenously and 500 mg per kg subcutaneously.

Reference: 1. Ikeda, Y. *et al.* J. Antibiotics (Japan) **3:** 726–729, 1950.

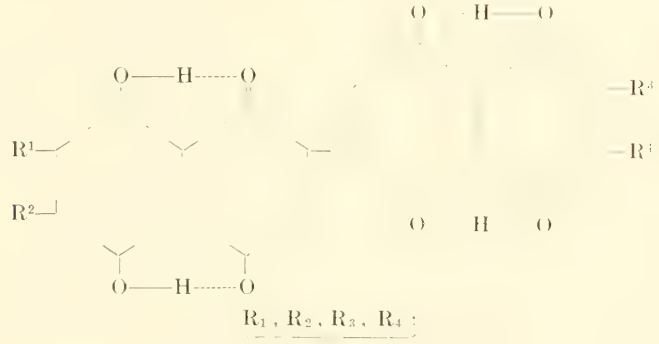
Actinorhodin

Produced by: Streptomyces coelicolor (2, 3). This organism produces other red pigments besides actinorhodin (1).

Method of extraction: Mycelium treated with *N* HCl for 15 minutes, filtered, exhaustively washed with methanol, then dried, ground in a mill with sand, and extracted with ether. Treatment with *N* NaOH gives a deep blue solution of the antibiotic (I). It is filtered; then acid is added to precipitate the antibiotic (II), which is extracted with acetone in a Soxhlet apparatus, then with dioxane under CO_2 (2).

Chemical and physical properties: Dinaphthazarin derivative (4). Probable formula $(C_{16}H_{14}O_7)_2$: C = 60.22%; H = 4.54%; O = 35.48%. Decomposes without melting at about 270°C. Thin, red needles (2) or microscopically fine prisms (3). Soluble in pyridine, piperidine, phenol, and al-

kali. Slightly soluble in alcohol, acetic acid, acetone, dioxane, tetrahydrofuran, and $NaHCO_3$ solution. Insoluble in petroleum ether, ether, carbon disulfide, carbon tetrachloride, and water. Ultraviolet absorption spectrum maxima at 523 and 560 mμ (dioxane), 573 and 641 mμ (concentrated H_2SO_4), and 588 and 641 mμ (NaOH). Red in acetic anhydride, becoming violet-blue with red fluorescence on addition of boric acid with warming. Red in dioxane. Deep blue in concentrated H_2SO_4, becoming red-violet on addition of boric acid. Bright blue in NaOH (1, 2). Forms a yellow crystalline triacetate (clustered needles), which gives no absorption of visible light and decomposes without melting at 260°C (2). In the mycelium, it exists mainly as protoactinorhodin (III). III is extracted from the dried mycelium with acetone, and precipitates as pink, microscopic prisms which turn red at 270°C and decompose at about 335°C (3). Partial structural formula of actinorhodin (4):



R_1, R_2, R_3, R_4:

2—COOH, 2—CH_3, $C_8H_{16}O_2$

Biological activity: Weakly active on *Staph. aureus* (2).

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Actinorubin

Produced by: A *Streptomyces* sp. that produces a red mycelium (1).

Synonym: Streptothricin-like substance.

Method of extraction: Adsorbed from broth-filtrate on Decalso at pH 7.0 and eluted with saturated NaCl. Eluate evaporated to dryness *in vacuo*. Residue extracted with boiling methanol. Cooling of extract precipitates crude actinorubin. Purified by chromatography on H_2SO_4-treated alumina, with 85 per cent methanol as solvent and developer. Precipitates from active fractions as helianthate (2).

Chemical and physical properties: Basic substance: $C_6H_{14}N_3O_2$ or $C_9H_{22}N_3O_4$. *HCl salt:* white powder. *Dihelianthate:* reddish orange needle clusters; m.p. 206–214°C. C = 51.40%; H = 5.93%; N = 17.36%; S = 7.34 or 8.87%. Soluble in water and methanol. Insoluble in ether. Positive biuret, Fehling (on boiling), and $KMnO_4$ tests. Negative Sakaguchi and Molisch tests. Dialyzable. Stable to boiling in aqueous solution at pH 6 to 7 for 15 minutes (2). Major component present in crude preparation indistinguishable by paper chromatography from pure streptothricin (wet butanol-*p*-toluenesulfonic acid) (4).

Biological activity: Active on gram-positive and gram-negative bacteria and mycobacteria. Limited activity on *B. cereus-mycoides* group and streptococci. Moderately active on *Trichophyton interdigitale*. Cross-resistance with lavendulin and streptothricin (1). Active *in vivo* (mice) on *K. pneumoniae* infections (3).

Toxicity: LD_{100} (mice) 73.9 mg per kg intraperitoneally (3).

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Actinoxanthine

Produced by: Streptomyces globisporus (3).

Method of extraction: Antibiotic salted out of broth-filtrate with $(NH_4)_2SO_4$. Precipitate dissolved in water and reprecipitated successively with $(NH_4)_2SO_4$. Chromatographed on Al_2O_3. Elution with water, acidification of active fractions to precipitate impurities, concentration, treatment with ion exchange resins, and precipitation of excess $CO_3^=$ with $BaSO_4$. Solution concentrated to dryness (1, 2).

Chemical and physical properties: Water-soluble, proteinaceous or peptide-like substance (1). Inactivated by air in dry form; more stable in aqueous solution (2).

Biological activity: Very active on gram-positive bacteria and tumors, including Ehrlich adenocarcinoma. No cross-resistance with penicillin, streptomycin, or chlortetracycline (1, 2). Active on cotton gummosis (4).

Toxicity: Maximal tolerated doses in mice (single administration): intraperitoneally, 0.025 mg per kg; intravenously, 0.023 mg per kg; subcutaneously and intramuscularly, 1.2 mg per kg; orally, 19.5 mg per kg (5).

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Actithiazic Acid

Produced by: Streptomyces sp. resembling *S. lavendulae* (1), *S. virginiae* (2), *S. acidomyceticus* (7), *S. cinnamonensis* (9), *S. lydicus* (13), and *S. roseochromogenes* (14).

Synonyms: Antibiotic PA 95 (15), mycobacidin (1), acidomycin (7), cinnamonin (9), thiazolidone antibiotic (12).

Method of extraction: I. Broth-filtrate extracted with n-butanol or butyl acetate at pH 2.0. Back-extracted into Na_2CO_3 solution. Extract adjusted to pH 4.5, then repeatedly extracted with butyl acetate. Butyl acetate evaporated, residue taken up in hot ethylene dichloride, treated with activated carbon, and filtered. Crystals form on cooling. Recrystallization from hot water, warm acetone, or methanol (4, 7, 14). II. Adsorbed from broth-filtrate on carbon. Eluted with ethanol-methanol (95:5) or 50 per cent aqueous acetone at pH 7.0, 50°C. Eluate adjusted to pH 8.0, filtered, and concentrated under reduced pressure. Concentrate extracted with butanol at pH 3.0, or with ethyl acetate at pH 1.0 to 2.0. Purified by chromatography on (a) silica gel with butanol as solvent and developer; active fractions washed with water at pH 2.0, concentrated and cooled to give a precipitate (5); or (b) alumina and developed with ethyl acetate, acetone, 1 per cent $Ba(OH)_2$, and 4 per cent NaOH, successively. Recrystallized from ethanol (10) IRA-400 and eluted with 10 to 20 per cent ammonium chloride. Eluate adjusted to pH 4.5 and freeze dried. Crystallized from chloroform with hexane. A hot ethylene dichloride solution of precipitate filtered through charcoal and cooled to give crystals. Recrystallized from methanol, and then water or ethylene dichloride (14).

Chemical and physical properties: Monobasic acid. Colorless needles; m.p. 137.5–141°C (1, 4, 5, 7, 10). Soluble in dilute NaOH, lower alcohols, ethyl acetate, benzyl alcohol, and hot water. Less soluble in acetone and distilled water. Very slightly soluble or insoluble in ether, ethylene dichloride, benzene, chloroform, petroleum ether, and water (5, 10). $[\alpha]_D^{25} = -60.5°$ (c = 1 per cent in absolute ethanol). End-absorption in ultraviolet light (5). Blue fluorescence in ultraviolet light (7). Infrared spectrum given in reference 5. $pK_a' = 5.8$. Negative 2,4-dinitrophenylhydrazine, Tollen, ninhydrin, and $FeCl_3$ tests (10). Positive bromine and $KMnO_4$ tests. A strongly alkaline solution heated with lead acetate darkens, with evolution of NH_3. Forms a white precipitate with $HgCl_2$. Stable (4). $C_9H_{15}O_3NS$ (5, 10). C = 49.86%; H = 6.86%; N = 6.46%; S = 14.4%. No C—CH_3. Actithiazic acid is 4-thiazolidone-2-caproic acid (5, 6). Structural formula is given in Chapter 6. Forms biologically active esters. *Methyl actithiazate:* Colorless needles; m.p. 53–54°C. $[\alpha]_D^{25} = -50.9$ (c = 1 per cent in methanol) (6). Oxidation with H_2O_2 yields pimelic acid (5); with HNO_3, adipic acid (7).

Biological activity: Active principally on mycobacteria and nocardiae; not active on other bacteria (2). Not active on tuberculosis infections in mice (3, 7). Activity reversed by biotin. Interferes with biotin synthesis in mycobacteria (2, 11). A number of esters formed by actithiazic acid are about twice as active as the parent compound on mycobacteria (6). The Na salt, methyl and ethyl esters, the hydrazide, and amide and guanidine derivatives of the antibiotic are active *in vitro* on mycobacteria (8).

Toxicity: LD_{50} (mice) 3.5 gm per kg intravenously and 20 gm per kg subcutaneously (7).

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Aklavin

Produced by: Streptomyces sp.

Method of extraction: I. Culture fluid and mycelium adjusted to pH 2.0, heated at 80°C for 10 minutes, cooled, and filtered. Mycelium retreated with HCl at pH 2.0 with heating. Combined extracts adjusted to pH 5.0 and extracted with chloroform. Chloroform extracts back-extracted with water at pH 2.0. II. Aqueous extract adjusted with NaOH to pH 9.0 under a stream of nitrogen and extracted with benzene. Benzene reextracted with water at pH 2.0. Process repeated once. Lyophilization of the final aqueous acidic extract yields crude aklavin hydrochloride.

Chemical and physical properties: Crude aklavin hydrochloride is very soluble in water, ethanol, chloroform, dioxane, and pyridine; soluble in acetone; and moderately soluble in ethyl acetate. Concentrated HCl solution is yellow; concentrated H_2SO_4 solution intensely reddish purple. A purplish pink color in alkaline solution is reversibly changed to yellow by $Na_2S_2O_4$. Addition of NH_4OH to an ethanol solution of the purple-blue magnesium acetate-aklavin complex produces a stable magenta-colored precipitate. Ultraviolet absorption maxima at 228, 258, and 288 mμ, and a visible light peak at 427 mμ. The crude antibiotic appears to contain several fractions, as shown by countercurrent distribution (ethyl acetate and 0.2 *M* phosphate buffer, pH 6.3). Melting points of crystalline salts: 168 or 188°C (picrate); 197°C (helianthate); 168 or 170°C (hydrochloride). Activity lost in alkaline solution on exposure to air; diffusibility in agar media doubled by heating at 80°C for 10 minutes. $C_{36}H_{40}O_{18}N_4$ (picrate): C = 52.80%; H = 4.97%; O = 35.22%; N = 6.85%. Formula of base: $C_{30}H_{37}O_{11}N$ or a multiple. Acid hydrolysis indicates that the antibiotic is made up of two moieties: a non-basic, water-insoluble moiety, and a colorless, water-soluble basic structure.

Biological activity: Active on a variety of bacteriophages; stimulates others. Some phages stimulated by aklavin in phage-host system are inhibited when free. Other phages that are completely inhibited in phage-host system are more resistant when free. Higher aklavin concentrations usually required for inactivation of free phage than phage in phage-host system. Active on some gram-negative and gram-positive bacteria, mycobacteria, fungi, and viruses. Moderately virucidal for eastern equine encephalomyelitis virus (EEE), slightly for PR 8 influenza, and not virucidal for MM virus or the Lansing strain. Inhibits Y-SK poliomyelitis virus in tissue culture. Active on EEE inoculated into mice when a mixture of equal volumes of virus (0.1 per cent) and antibiotic (0.01 mg per ml) stood for 1 hour before inoculation.

Toxicity: LD_0 (mice) 150 mg per kg intravenously; 100 mg per kg given intraperitoneally is not tolerated.

Reference: 1. Strelitz, F. *et al.* J. Bacteriol. **72:** 90–94, 1956.

Alazopeptin

Produced by: Streptomyces griseoplanus (2).

Synonym: Distantly related to azaserine and DON, which are diazo derivatives of amino acids. Alazopeptin contains diazo groups in a peptide (1).

Method of extraction: Broth stirred with activated carbon. Antibiotic eluted with 50 per cent acetone. Eluates concentrated *in vacuo*, residual solution adjusted to pH 9.0 and extracted with butanol containing 2 per cent Arquad 2C. Back-extracted into water at pH 2.0 to 2.5. Aqueous

extract neutralized to pH 7.0, concentrated *in vacuo*, and freeze dried. Purification from a methanol solution on alumina. Developed with methanol and 90 per cent methanol. Further purification by partition chromatography on Celite 545 with development with a lower phase of CCl_4-90 per cent aqueous phenol-water, 82:18:25. Peak fractions are pooled, diluted with carbon tetrachloride, and extracted with water. Aqueous extract adjusted to pH 7.0 and extracted with ether. Extract concentrated *in vacuo* and freeze dried. Recrystallization from 95 per cent ethanol in the cold, 90 per cent aqueous methanol, and 70 per cent aqueous acetone (1).

Chemical and physical properties: $C_{15}H_{21}N_7O_6 \cdot H_2O$: C = 43.59%; H = 5.96%; N = 23.64% (Dumas); N (diazo) = 13.72%; N (nondiazo) = 10.4%; H_2O = 2.85%. No specific melting point. Decomposes over a wide temperature range. Very soluble in water; somewhat soluble in acetic acid, formamide, dimethyl sulfoxide, and aqueous solutions of methanol, ethanol, and acetone. Insoluble in absolute alcohols, acetone, ethyl acetate, and ether. $[\alpha]_D^{25} = +9.5°$ (c = 4.7 per cent in water). Stable at alkaline pH and neutrality, but not at acid pH. Ultraviolet absorption maxima (pH 7.0, phosphate buffer) at 242 mμ ($E_{1cm}^{1\%}$ 321) and 274 mμ ($E_{1cm}^{1\%}$ 549), typical of aliphatic diazoketones. Infrared spectrum given in reference 1. Liberates nitrogen on acidification with strong acids, with loss of biological activity and ultraviolet absorption characteristics. Positive ninhydrin reaction. Positive biuret reaction after acidification or treatment with bromine water. Untreated alazopeptin gives a yellow precipitate with the biuret reagents. Acid hydrolysis products include α-alanine, representing 21.4 per cent of the antibiotic. Oxidation with periodic acid yields a peptide, which on acid hydrolysis yields glutamic acid and α-alanine, indicating that the antibiotic is a peptide containing α-alanine and a C_6 diazoketoamino acid (1).

Biological activity: Slightly active in mice on sarcoma 180 (ascitic and solid) and Ehrlich carcinoma (solid form) (4).

Toxicity: LD_{50} (rat) 150 mg per kg intraperitoneally. Toxic to fetuses *in utero* at one thirtieth of the adult LD_{50} (3).

References:

1. De Voe, S. E. *et al.* Antibiotics Ann. 730–735, 1956–1957.
2. Backus, E. J. *et al.* Antibiotics & Chemotherapy **7:** 532–541, 1957.
3. Thiersch, J. B. Proc. Soc. Exptl. Biol. Med. **97:** 888–889, 1958.
4. Sugiura, K. Ann. N. Y. Acad. Sci. **76:** 575–585, 1958.

Albofungin

Produced by: Streptomyces albus (2) or *S. albus* var. *fungatus* (3).

Method of extraction: Mycelium washed with acetone, then extracted with *N* HCl in acetone. Extract neutralized with $CaCO_3$; precipitate forms on dilution with water. Reprecipitated from acidic acetone with petroleum ether. Chromatographed on alumina from acetone (2).

Chemical and physical properties: Complex, containing several closely related components. Yellow powder. Decomposes without melting at 190°C. Soluble in chloroform, acetone, and dimethylformamide. Less soluble in alcohols. Insoluble in water and ether. Ultraviolet absorption spectrum maxima at 240, 255, 305, and 375 mμ. Contains C, H, O, and N (2).

Biological activity: Active on yeasts, filamentous fungi, gram-positive bacteria (1), and certain gram-negative bacteria (3).

References:

1. Caykovskaya, S. M. and Tyebyakina, A. E. Abstr. Communs. Symposium on Antibiotics. Prague, 1959, pp. 142–143.
2. Chochlov, A. S. and Liberman, G. S. Abstr. Communs. Symposium on Antibiotics. Prague, 1959, pp. 154–155.
3. Solovyeva, N. J. *et al.* Abstr. Communs. Symposium on Antibiotics. Prague, 1959, pp. 189–191.

Albomycetin

Produced by: Streptomyces albus (1) and a *Streptomyces* sp. resembling *S. albus* (3).

Method of extraction: I. Broth-filtrate extracted with ethyl acetate, benzene, chloroform, or butanol at pH 8.0. Back-extracted into acidic water (pH 1.0). The aqueous extract can be: (a) concentrated and adjusted to alkaline pH to precipitate the antibiotic; or (b) extracted into ethyl acetate at pH 8.0 and chromatographed on Al_2O_3 with ethyl acetate as developer. Colorless fraction dried *in vacuo*, taken up in benzene, and again dried *in vacuo*. Recrystallized from acetone-ether (1:9) (1, 3). II. Adsorbed from broth-filtrate on a cation exchange resin or activated charcoal. Eluted with 80 per cent acidic acetone. Eluate concentrated *in vacuo*, then treated as in I(b) (1, 3).

Chemical and physical properties: Basic substance. Colorless hexagonal crystals; m.p. 166–167°C (1) or 164–166°C (3). Soluble in xylene,

benzene, ethylene dichloride, methanol, ethanol, benzyl alcohol, and water at acid pH. Soluble at high temperatures in acetone, ethyl acetate, dioxane, butanol, and Tetralin. Scarcely soluble in ether, carbon bisulfide, distilled water, and petroleum ether. No characteristic ultraviolet absorption in ethyl ether or ethanol. Infrared data given in reference 1, and spectrum in reference 3. $[\alpha]_D^{22} = -48.7°$ (c = 1 per cent in chloroform). Positive Fehling, Tollen, and silver mirror tests. Cherry-red color with Elson-Morgan reagent. Negative biuret, ninhydrin, Millon, Molisch, orcinol, phloroglucinol, Pauly, $FeCl_3$, and Sakaguchi tests. Stable to boiling at pH 2 to 9 for 10 minutes. Most stable at neutrality, less so at acid pH, and relatively unstable at alkaline pH. Precipitated by ammonium reineckate and picric acid, but not flavianic acid or methyl orange. $C_{25}H_{44}NO_7$: C = 63.38%; H = 9.14%; N = 2.91%; molecular weight 477; or $C_{32}H_{54}O_9N$: molecular weight 586.4. No S, P, or halogen (1, 3).

Biological activity: Active on gram-positive bacteria at 0.1 to 12.5 μg per ml, including *M. smegmatis*. Less active (25 to 250 μg per ml) on other mycobacteria. Inactive on gram-negative bacteria, filamentous fungi, yeasts, phage, and viruses tested. Active *in vivo* against pneumococcal and *Borrelia duttonii* infections (1, 3). Cross-resistance with erythromycin-carbomycin group (2).

Toxicity: LD_{50} (mice) 1.5 gm per kg orally, 0.5 gm per kg subcutaneously (3). Mice tolerate 249 mg per kg intraperitoneally, 124.5 mg per kg intravenously, and 41.5 mg per kg intracerebrally. Fifty per cent are killed by 332 mg per kg intraperitoneally, 166 mg per kg intravenously, and 58 mg per kg intracerebrally (1).

References:

1. Takahashi, B. J. Antibiotics (Japan) **7A:** 149–154, 1954.
2. Nishimura, H. *et al.* Ann. Rept. Shionogi Research Lab. **6:** 278–285, 1956 (Chem. Abstr. **51:** 5193d, 1957).
3. Kuroya, M. and Nishimura, H. Japanese Patent 7750, September 17, 1957.

Alboverticillin

Produced by: Streptomyces sp.

Chemical and physical properties: Colorless, amorphous powder. Soluble in water and methanol. Insoluble in acetone, esters, and ether. Ultraviolet absorption spectrum maximum at 208 mμ (0.1 N HCl) or 218 mμ (0.1 N NaOH). $[\alpha]_D^{20} = -33.5°$ (c = 1.0 per cent in water). Negative Tollen, Molisch, Benedict, maltol, Elson-Morgan, biuret, Millon, Sakaguchi, anthrone, and $FeCl_3$ tests. Yellow precipitate with Fehling's reagent; positive ninhydrin. Stable to heating at 100°C for 1 hour at acid and neutral pH. Does not yield furfural on acid hydrolysis. *Reineckate:* C = 32.03%; H = 5.21%; N = 22.72%; Cr = 9.35%. *HCl:* C = 40.58%; H = 6.48%; N = 18.12%; Cl = 8.98%. No S.

Biological activity: Active on mycobacteria (0.002 to 2.0 μg per ml). Very slightly active on *Sarcina lutea*, *B. subtilis*, and *B. anthracis*. Not active on *Staph. aureus* or other bacteria, fungi, or yeasts tested.

Toxicity: LD_{50} (mice) 50 to 100 mg per kg intravenously. No delayed toxicity.

Reference: 1. Maeda, K. *et al.* J. Antibiotics (Japan) **11A:** 30–31, 1958.

Aliomycin

Produced by: Streptomyces acidomyceticus.

Remarks: This culture also produces actithiazic acid. Culture belongs to the *lavendulae* group.

Method of extraction: Extracted from the mycelium with hot methanol. Extract concentrated *in vacuo*, adjusted to pH 9.0, and extracted with butanol. Solvent removed *in vacuo*, and acetone added to the residual syrup to give aliomycin. Can also be extracted from mature culture-broths.

Chemical and physical properties: Pentaene. Yellow powder. Soluble in distilled water, alkaline water, glacial acetic acid, methyl Cellosolve, ethylene glycol, methanol, ethanol and hot butanol, isoamyl alcohol, and dioxane. Insoluble in water at pH 3.0, or in ether, benzene, ethyl acetate, or acetone. Ultraviolet absorption spectrum maxima at 321, 330, and 350 mμ. Gives a dark purple color in concentrated H_2SO_4. Positive Fehling test. Slightly positive Molisch test. Stable at neutrality. Contains S and N, but no halogen.

Biological activity: Active on fungi and yeasts at 7.5 to 20 μg per ml. Active on *Trichomonas foetus*. Active on Yoshida sarcoma cells at 0.5 mg per ml. Activity partially reversed by cysteine.

Toxicity: LD_{50} (crude substance, mice) 45 mg per kg intraperitoneally; 2.65 gm per kg orally.

Reference: 1. Igarashi, S. *et al.* J. Antibiotics (Japan) **9B:** 101–103, 1956.

Alomycin

Produced by: Streptomyces sp. (1).

Method of extraction: Dried powdered mycelium extracted with ethyl acetate containing about 1

per cent triethylamine, methanol-1 per cent triethylamine, acetone, or an ethyl acetate-acetone-water mixture (5:1:0.5). Ethyl acetate-extracts are concentrated *in vacuo*. To the resulting oily brown residue is added a saturated solution of tannin to precipitate impurities, and the whole filtered. Water is added to the filtrate and mixed, and the aqueous and organic phases separated. To the aqueous phase NaCl is added to saturation, to salt out an additional amount of the organic phase. Both organic phases are extracted with methanol, extract concentrated *in vacuo*, and lyophilized to give a brown oil (3).

Chemical and physical properties: Light brown clear oil. Ultraviolet absorption spectrum maximum at 270 mμ, with a small peak at 350 mμ (methanol). Does not form active precipitates with picric acid, *p*-toluenesulfonic acid, methyl orange, ammonium reineckate, resorcinol, or procaine HCl. Rf values in various systems of paper chromatography are given, and are said to differentiate the antibiotic from those given in the key system of Ammann and Gottlieb (4). Unstable at acid pH; most stable at pH 6.0; 50 per cent inactivated after 3 hours at 100°C (3).

Biological activity: Active on *Candida*. Not active on bacteria (2).

References:

1. Woznicka, W. *et al.* Med. Doświadczalna i Mikrobiol. **9:** 57–62, 1957.
2. Woznicka, W. *et al.* Med. Doświadczalna i Mikrobiol. **9:** 293–308, 1957.
3. Woznicka, W. *et al.* Med. Doświadczalna i Mikrobiol. **9:** 441–450, 1957.
4. Ammann, A. and Gottlieb, D. Appl. Microbiol. **3:** 181–186, 1955.

Althiomycin

Produced by: Streptomyces althioticus (1).

Method of extraction: Broth adjusted to pH 6.0 and filtered. Filtrate extracted with butyl acetate or butanol. Extracts concentrated *in vacuo*. Cooling of residue precipitates althiomycin. Recrystallized from ethyl Cellosolve. Can be adsorbed from broth on clay and eluted with aqueous acetone. Can also be extracted with ethanol, acetone, or ethyl acetate from mycelium.

Chemical and physical properties: White needles; brown at 120–160°C and decompose at 172–174°C. Soluble in ethyl Cellosolve, dioxane, and pyridine. Slightly soluble in acetone, methanol, butanol, ethyl acetate, and butyl acetate. Insoluble in water, ether, petroleum ether, and benzene. $[\alpha]_D^{20} = +20.3°$ (c = 1.33 per cent in methyl Cellosolve). Ultraviolet absorption spectrum maximum at 220 to 223 mμ ($E_{1cm}^{1\%}$ 810) and a shoulder at 285 to 290 mμ ($E_{1cm}^{1\%}$ 210) in 0.03 *N* HCl containing 1 per cent methyl Cellosolve, or at 235 mμ ($E_{1cm}^{1\%}$ 611) and 300 to 305 mμ ($E_{1cm}^{1\%}$ 317) in 0.03 *N* NaOH containing 1 per cent methyl Cellosolve. Infrared spectrum given in reference 1. Positive ninhydrin and Tollen tests. Negative $FeCl_3$, Fehling, and Sakaguchi reactions. $C_{15}H_{14}N_4S_2O_6$: C = 44.49%; H = 3.51%; N = 13.92%; S = 14.77%. Stable at pH 5 to 7.

Biological activity: Active on gram-positive and some gram-negative bacteria, but not on mycobacteria, *Pr. vulgaris*, *Ps. aeruginosa*, fungi, or yeasts. Active in mice on *D. pneumoniae* and *Sal. typhosa*.

Toxicity: Mice tolerate 720 mg per kg intraperitoneally.

Reference: 1. Yamaguchi, H. *et al.* J. Antibiotics (Japan) **10A:** 195–200, 1957.

Amaromycin

Produced by: Streptomyces flavochromogenes.

Synonym: Related to picromycin and griseomycin.

Method of extraction: Broth, adjusted to pH 7.5, is extracted with ⅓ volume of benzene. Benzene is extracted three times with pH 2.0 HCl solution. HCl solution adjusted to pH 5.7 and back-extracted twice with benzene. Benzene is dried *in vacuo* and the crude residue taken up in hot CS_2. When cooled, white prisms precipitate out. Recrystallization from ethanol.

Chemical and physical properties: Basic, bitter-tasting prisms; m.p. 164.5–165°C. $[\alpha]_D^{25} = +6.19°$ (1 per cent in ethanol). Free base: C = 63.66%; H = 8.73%; N = 3.0%; O = 24.61%. $C_{25}H_{39}O_7N$. Ultraviolet spectrum shows broad peak at approximately 220 mμ. Infrared spectrum (CCl_4 as solvent) shows bands at 2.9, 3.4, 5.7, 6.1, 6.85, 7.2, 7.45, 7.85, 8.38, 8.60, 9.0, 9.25, 9.5, 10.15, 10.60, and 11.3 μ. Readily soluble in ether, chloroform, and methanol. Soluble in benzene, toluene, ethyl acetate, butanol, carbon tetrachloride, ethanol, and warm carbon disulfide. Slightly soluble in cold CS_2. Scarcely soluble in petroleum ether and water. A yellow color develops when concentrated H_2SO_4 is added to an aqueous solution of the antibiotic. Positive Fehling and Tollen tests. Negative $FeCl_3$, Schiff, Sakaguchi, xanthoproteic, biuret, ninhydrin, and Molisch tests. Precipitated from aqueous solution by picric acid and reineckate salt; stable to heating at 100°C for 20 minutes at pH 2 and pH 7, and for 10 minutes at pH 8.

Biological activity: Activity limited to gram-

positive and a few gram-negative bacteria, such as members of the genera *Hemophilus* and *Brucella*. Not active on gram-negative bacteria, yeasts, or fungi. Slightly active on some saprophytic mycobacteria.

Toxicity: LD_{50} (mice) 150 to 232 mg per kg intravenously.

Reference: 1. Hata, T. *et al.* J. Antibiotics (Japan) **8A:** 9–14, 1955.

Amicetin

Produced by: Streptomyces vinaceus-drappus (1, 3, 7), *S. fasciculatis* (1, 3), *S. sacromyceticus* (6), *S. sindenensis* (8, 11), *S. plicatus* (12), and a *Streptomyces* sp. resembling *S. griseus* (9).

Synonyms: Sacromycin (9), allomycin (8), antibiotic D 13.

Remarks: An original description of this antibiotic indicated the presence of more than one antibiotic in the broth of *S. vinaceus-drappus*. It is possible that some of these were synonymous with amicetin B (plicacetin) and bamicetin (7).

Method of extraction: I. Adsorbed from broth-filtrate on a cation exchange resin (IRC-50, IR-100, etc.) or on charcoal. Eluted from charcoal with 10 per cent aqueous acid-acetone. Eluates adjusted to pH 7 to 8 and freeze dried. Purified by countercurrent distribution (water-methylene chloride or water-butanol). Anhydrous crystals can also be prepared by precipitation from anhydrous methanol (7).

II. Clarified broth extracted with butanol at pH 8.5 to 9.5. Butanol extracted with dilute sulfuric acid (final pH 2.0). This is repeated and adjustment to pH 8.5 with seeding precipitates the free base. Further purification is obtained by treating a dilute HCl solution with activated carbon, filtering, and reprecipitating the antibiotic from solution by adjusting to pH 8 to 8.5. The slurry of hydrated needles is converted to a granular, high melting point form by stirring at 60–65°C (3).

Chemical and physical properties: Amphoteric substance. *Base:* colorless. Exists in two forms: needles, m.p. 165–169°C, or granular, m.p. 244–245°C. Soluble in aqueous mineral acid and alkali and water-saturated butanol. Slightly soluble in water at 22°C. Ultraviolet maxima at 306 mμ ($E^{1\%}_{1cm}$ 512) in 50 per cent aqueous ethanol; 272 mμ ($E^{1\%}_{1cm}$ 283) and 325 mμ ($E^{1\%}_{1cm}$ 412) in 50 per cent ethanol-50 per cent 0.1 *N* NaOH; 304 mμ ($E^{1\%}_{1cm}$ 451) in 50 per cent ethanol-50 per cent 0.1 *N* HCl; 305 mμ ($E^{1\%}_{1cm}$ 465) in water; 316 mμ ($E^{1\%}_{1cm}$ 433) in 0.1 *N* HCl; and 322 mμ ($E^{1\%}_{1cm}$ 470) in 0.1 *N* NaOH. Infrared spectrum given in reference 3. $[\alpha]_D^{24} = +116.5°$ (c = 0.5 per cent in 0.1 *N* HCl). Stable in alkaline solution at >pH 8.0; stable in acid. Rf values on paper chromatography given in reference 3. $C_{29}H_{42}N_6O_9$: C = 55.98%; H = 6.92%; N = 13.18%. Molecular weight about 640. pK_a' about 1.1, 7.0, and 10.4. Mild alkaline hydrolysis yields cytosamine. $C_{18}H_{32}N_4O_6$. Acid hydrolysis yields a salt of a base, cytimidine, $C_{15}H_{17}N_3O_4$; m.p. (of salt) 264–266°C. Structural formula of amicetins (3, 4, 7, 10) given in Chapter 6. The dimethylamino sugar is amosamine. *Hydrochloride:* Fine white crystals; m.p. 190–192°C. More water-soluble than amicetin. Methyl orange and orange II react with amicetin to give water-insoluble salts. *Orange II salt:* m.p. 204–206°C.

Biological activity: Both needles and granular forms have similar microbiological activity, being active largely against gram-positive bacteria, especially mycobacteria (2, 4). Active in protecting mice infected with *M. tuberculosis* H37Rv (1). Prolong survival time of mice with transmitted leukemia (Line 82), but not in mice with two other leukemia strains (5).

Toxicity: Citrate complex of amicetin: acute LD_{50} (mice) about 90 mg per kg intravenously, 600 to 700 mg per kg subcutaneously. LD_{50} (rats) about 200 mg per kg intravenously. Especially toxic to guinea pigs, being 40 times as toxic as streptomycin, but only $\frac{1}{10}$ as toxic as penicillin given subcutaneously (2).

References:

1. McCormick, M. H. and Hoehn, M. M. Antibiotics & Chemotherapy **3:** 718–720, 1953.
2. DeBoer, C. *et al.* J. Am. Chem. Soc. **75:** 499, 1953.
3. Hinman, J. W. *et al.* J. Am. Chem. Soc. **75:** 5864–5866, 1953.
4. Flynn, E. H. *et al.* J. Am. Chem. Soc. **75:** 5867–5871, 1953.
5. Burchenal, J. H. *et al.* Proc. Soc. Exptl. Biol. Med. **86:** 891–893, 1954.
6. Nisho, Y. *et al.* Japan. J. Bacteriol. **9:** 600–601, 1954.
7. British Patent 708,686, May 5, 1954.
8. Tatsuoka, S. *et al.* Ann. Rept. Takeda Research Lab. **13:** 41–44, 1954.
9. Hinuma, Y. *et al.* J. Antibiotics (Japan) **8A:** 148–152, 1955.
10. Stevens, C. L. *et al.* J. Am. Chem. Soc. **78:** 6212, 1956.
11. Nakazawa, K. and Fujii, S. Ann. Rept. Takeda Research Lab. **16:** 109–110, 1957.
12. Haskell, T. H. *et al.* J. Am. Chem. Soc. **80:** 743–747, 1958.

Amicetin B

Produced by: Streptomyces plicatus (2, 3).

Synonyms: Plicacetin, believed to be a precursor of amicetin (1); antibiotic C (2).

Method of extraction: Broth-filtrate extracted with 1-butanol at slightly alkaline pH values, extract concentrated, then back-extracted into water at pH 2.0. Could be precipitated from aqueous solutions with various aromatic azosulfonic acid dyes, or adsorbed on and eluted from IRC-50. Precipitated from absolute methanol solution with ether or by raising an aqueous solution to pH 9.0 with NH_4OH. Recrystallization from hot water or dilute aqueous methanol gives crystal form I; from ethyl acetate, crystal form II; and from absolute ethanol, crystal form III (1).

Chemical and physical properties: Crystal I: m.p. 182–184°C; colorless needles. *Crystal II:* m.p. 160–163°C; colorless needles. *Crystal III:* m.p. 222–225°C; dense prisms. *Base:* $[\alpha]_D^{26} = +181°$ (c = 2.7 per cent in methanol). Soluble in dilute acid, lower alcohols, chloroform, and methylene chloride. Sparingly soluble in ethyl acetate, ether, and cold water. Insoluble in benzene and petroleum ether. Ultraviolet absorption spectrum maxima: 257 and 311.5 mμ (0.1 *N* HCl), 249 and 321 mμ (pH 7.0, phosphate buffer), 329 mμ (0.1 *N* NaOH). pK_a = 2.2, 7.0, and 10.9. Infrared spectrum of crystal III given in references 1 and 3. Rf value = 0.86 (1-butanol saturated with 0.05 *M* pH 7.0 phosphate buffer) (3). Rf (n-butanol and acetic acid) = 0.28 (2). Soluble in dilute acid. Negative Molisch, biuret, Sakaguchi, ninhydrin, $FeCl_3$, and Fehling tests. Positive Ehrlich and Bratton-Marshall tests (both for arylamino groups) (2). Gives insoluble precipitates with picric, picrolonic, styphnic, and phosphotungstic acid. A hydrochloric acid solution reacts with nitrous acid giving a diazo compound (I). This reacts with N-(1-naphthyl)ethylenediamine to give a violet color with a maximum at 550 mμ. Alkaline hydrolysis products include cytosamine. Acid hydrolysis product is *p*-aminobenzoylcytosine. $C_{25}H_{35}N_5O_7$: C = 57.69%; H = 6.84%; N = 13.52% (1). Structural formula given in Chapter 6.

Biological activity: Active on gram-positive bacteria, including mycobacteria. Negligible activity on gram-negative bacteria and fungi. Has less activity *in vitro* and *in vivo* on *M. tuberculosis* H37Rv than amicetin or bamicetin (3).

References:

1. Sensi, P. *et al.* Antibiotics & Chemotherapy **7:** 645–652, 1957.
2. British Patent 707,332, April 14, 1954.
3. Haskell, T. H. *et al.* J. Am. Chem. Soc. **80:** 743–747, 1958.

Amidomycin

Produced by: Streptomyces sp. (1).

Synonym: Resembles valinomycin (1, 2).

Method of extraction: Broth and mycelium treated with a filter-aid, the pH adjusted to 3.5 with concentrated HCl, and filtered. The solid residue is extracted with methanol. Methanol removed by evaporation *in vacuo* at 40°C; the residual suspension diluted with water and freeze dried. Solid extracted in a Soxhlet apparatus for 3 to 6 hours with petrol (b.p. 30–60°C). Petrol evaporated to dryness, giving an oily residue which partially crystallizes on standing. Recrystallization from petrol or 50 per cent aqueous ethanol. Increased yields can be obtained by chromatographing benzene solutions of the dried mother liquors on silicic acid and developing with benzene, then chloroform. In large fermenters, most of the activity is in the broth (1).

Chemical and physical properties: Neutral, colorless needles; m.p. 192°C. $[\alpha]_D^{25} = +19.2°$ (c = 1.2 per cent in ethanol). Insoluble in water; partially soluble in petrol; very soluble in organic solvents. Stable. No characteristic absorption in ultraviolet or visible light. Infrared absorption spectrum given in reference 2. Hydrolysis in alcoholic acid or alkali, followed by treatment with concentrated HCl, gives two products, D(−)-valine and D(−)-α-hydroxyisovaleric acid, both biologically inactive. Rf values of 0.86 (paper impregnated with ethylene glycol, with petrol, b.p. 100–120°C as mobile phase); 0.90 (water-saturated n-amyl alcohol); 0.36 (ethyl alcohol-acetic acid-water, 3:1:6); and 0.89 (ethanol-water, 2:3). C = 60.22%; H = 8.62%; N = 7.06%; C-Me = 31.7%. $C_{40}H_{68}O_{12}N_4$. Chemical structure shown at top of p. 187.

Biological activity: Active on yeasts and filamentous fungi. Resistance does not develop readily. Not active on bacteria (1).

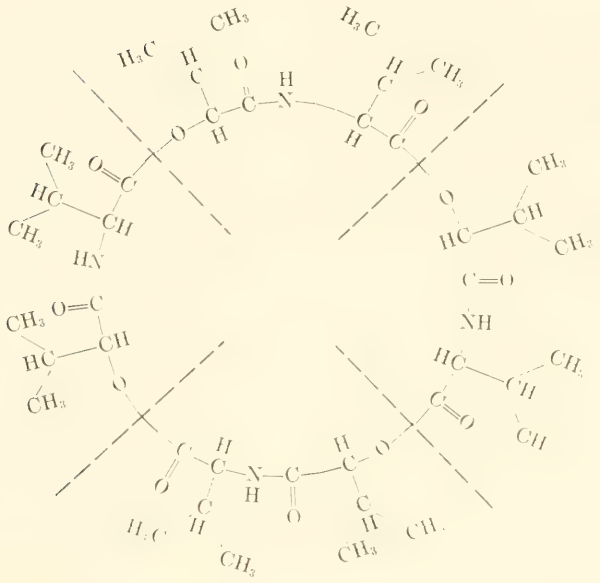
References:

1. Taber, W. A. and Vining, L. C. Can. J. Microbiol. **3:** 953–965, 1957.
2. Vining, L. C. and Taber, W. A. Can. J. Chem. **35:** 1109–1116, 1957.

Amphomycin

Produced by: Streptomyces canus (1, 3), *S. violaceus* (5), and a *Streptomyces* sp. resembling *S. lavendulae* (5).

Synonym: Closely related to crystallomycin and aspartocin.



Method of extraction: Broth-filtrate adjusted to pH 1.95 and filtered. Filtrate extracted with n-butanol, washed with water at pH 2.0, then back-extracted into water at pH 6.4 to 7.4. Procedure repeated (3). I. Water-extracts from butanol treated with Darco G-60 at pH 5 to 7 and eluted with butanol-saturated water (pH 9). Re-extracted into butanol. Extract concentrated *in vacuo* and ether added to precipitate amphomycin. Reprecipitated from anhydrous ether (5). II. Water-extracts freeze dried, taken up in water, adjusted to pH 2.0 with phosphoric acid, and extracted into butanol. Extract filtered and decolorized with activated charcoal. Butanol concentrated *in vacuo*. Amphomycin precipitated on addition of excess ethyl acetate. Can also be precipitated from an aqueous solution (pH 2.2) by isoelectric precipitation at pH 3.4. III. Purified by precipitation as the reineckate or as the calcium salt (reaction of calcium chloride with Na amphomycin) (1, 3).

Chemical and physical properties: Amphoteric polypeptide with an isoelectric point at 3.4 to 3.5. White to off-white amorphous powder. Soluble in water. Soluble in methanol as the acid form and as the salt form. Soluble in higher alcohols containing at least four carbon atoms, only in the acid form. Insoluble in nonpolar solvents. End-absorption (210 to 230 mμ) of ultraviolet light. Infrared spectrum given in reference 5. $[\alpha]_D^{25} = +7.5°$ (c = 1 per cent in water at pH 6.) Optical activity decreases at higher or lower pH. Positive biuret test. Negative ninhydrin, Sakaguchi, Molisch, Ehrlich-Pauly, xanthoproteic, Adamkiewicz, Ehrlich, Liebermann, and Seliwanoff tests. Stable in aqueous solution for at least 1 month at room temperature. Rf = 0.09 on paper chromatography (water-saturated butanol-collidine-β-naphthalenesulfonic acid, 98:2:1). C = 54.4%; H = 7.2%; N = 14.2% (for the free acid). Minimal molecular weight 1400 to 1500. Acid hydrolysates contain aspartic acid, glycine, valine, proline, and a fifth unidentified amino acid (1, 3, 5).

Biological activity: Active on gram-positive bacteria. Not active on gram-negative bacteria, *C. albicans*, or *Trichophyton mentagrophytes* (1, 3). Active *in vivo* (mice) on *D. pneumoniae* (1, 3), *B. anthracis*, *Erysipelothrix rhusiopathiae* (5), *Trypanosoma gambiense*, and *T. rhodesiense* infections (4).

Active on downy mildew of cucumber, *Pseudoperonospora cubensis* (6).

Toxicity: LD_{50} (mice) 177.8 mg per kg intravenously. LD_{50} (dogs) 100 mg per kg intravenously (Na salt) (2).

Utilization: Possible use in topical application in gram-positive infections.

References:

1. Heinemann, B. *et al.* Antibiotics & Chemotherapy **3**: 1239–1242, 1953.
2. Tisch, D. E. *et al.* Antibiotics Ann. 1011–1019, 1954–1955.
3. British Patent 736,325, September 7, 1955.

4. Packchanian, A. Antibiotics & Chemotherapy 6: 684–691, 1956.
5. Giolitti, G. *et al.* Giorn. microbiol. 3: 70–83, 1957.
6. Ark, P. A. and Thompson, J. B. Plant Disease Reptr. 41: 452–454, 1957.

Amphotericin A

Produced by: Streptomyces sp. The same culture produces amphotericin B.

Method of extraction: Whole culture mixed with an equal volume of isopropanol; pH adjusted to 10.5. After removal of the mycelium, solution is concentrated *in vacuo*, with formation of a precipitate. Precipitate washed with water and acetone before drying. This crude concentrate contains both amphotericin A and B. Crude material slurried in a 2 per cent (weight per volume) methanol solution of calcium chloride. Amphotericin A is dissolved by the methanolic calcium chloride solution and can be precipitated from this solution by the addition of water. Repeating this latter procedure yields crystalline amphotericin A.

Chemical and physical properties: Conjugated tetraene. Slightly soluble (8 to 40 mg per ml) in methanol, glacial acetic acid, and propylene glycol. Soluble in N,N-dimethylformamide, methanol-calcium chloride, and basic methanol to the extent of 90 to 125 mg per ml. Insoluble in water, ethanol, butanol, acetone, ethyl acetate, ether, and benzene. Decomposition above 153°C. $[\alpha]_D^{25.5}$ = −9.9° in 0.1 *N* methyl hydroxide-hydrochloric acid. $[\alpha]_D^{23.5}$ = +32° in acid dimethylformamide. C = 60.3%; H = 8.4%; N = 1.7%. The ultraviolet light absorption of amphotericin A is characteristic of a conjugated tetraene, and is similar to that of rimocidin and nystatin. Amphotericin A is amphoteric and forms a water-soluble sodium salt. Negative $FeCl_3$ test; positive Molisch test. Potassium permanganate and bromine carbon tetrachloride solutions decolorized.

Biological activity: Active *in vitro* against fungi but not against bacteria. Amphotericin A has a wide spectrum of antifungal activity, being active against both filamentous fungi and yeasts. Active *in vivo* against experimental *C. albicans*, *Histoplasma capsulatum*, and *Cryptococcus neoformans* infections of mice. Absorbed after oral administration to mice.

Toxicity: LD_{50} (mice) 450 mg per kg intraperitoneally.

Reference: 1. Steinberg, B. A. *et al.* Antibiotics Ann. 574–591, 1955–1956.

Amphotericin B

Produced by: Streptomyces nodosus (15). This culture also produces amphotericin A (1).

Method of extraction: Whole culture mixed with an equal volume of isopropanol; pH adjusted to 10.5. After removal of the mycelium, solution is neutralized and concentrated *in vacuo*, with formation of a precipitate. Precipitate washed with water and acetone before drying. This crude concentrate contains both amphotericin A and B. Crude material slurried in a 2 per cent (weight per volume) methanol solution of calcium chloride. Amphotericin B is not appreciably dissolved in this solvent. Previously undissolved material extracted with acidified N,N-dimethylformamide. Methanol added to solution. Reaction of the formamide-methanol solution maintained at pH 5, while water is added, resulting in precipitation of amphotericin B. Repeating the latter procedure yields crystalline amphotericin B (1).

Chemical and physical properties: Amphoteric conjugated heptaene. Deep yellow prisms (from dimethylformamide). Decomposes at >170°C. Soluble in dimethyl sulfoxide and acetic N,N-dimethylformamide (60 to 80 mg per ml). Slightly soluble (0.2 to 4 mg per ml) in propylene glycol, glacial acetic acid, and N,N-dimethylformamide. Insoluble in water, methanol, ethanol, butanol, acetone, ethyl acetate, ether, benzene, chloroform, pyridine, and alcoholic KOH. λ_{max}^{MeOH}, 363, 382, 406. Infrared spectrum is given in reference 1. $[\alpha]_D^{23.5}$ = −33.6 (in 0.1 *N* methyl hydroxide-HCl) or +33.3 (in acid dimethylformamide). Positive Molisch, $KMnO_4$, and Br-CCl_4 tests. Negative $FeCl_3$ test. $C_{46}H_{73\text{-}75}NO_{18\text{-}20}$: C = 57.59%; H = 8.0%; N = 1.7%. Neutralization equivalent, 959 (perchloric acid in acetic acid). Possibly contains a lactone group. Hydrogenation product is colorless, biologically inactive, tetradecahydroamphotericin B, $C_{46}H_{87}NO_2$, having an infrared spectrum similar to the parent polyene and which darkens at 160°C but is not decomposed at 250°C. Prolonged acetolysis of amphotericin B yields tri- and tetraacetates of an amino desoxyhexose "mycosamine," $C_8H_{13}NO_4$, which is also obtained from nystatin (see Chapter 6). Amphotericin B forms a poorly water-soluble sodium salt (1, 2, 5, 9).

Biological activity: Active *in vitro* against fungi but not against bacteria. Amphotericin B is more active against yeasts and yeast-like fungi than against filamentous fungi (1, 16). Active in tissue culture at 0.1 to 1.0 μg per ml against the pathogenic form of *Histoplasma capsulatum*, *Sporotrichium schenkii*, *Cryptococcus neoformans*, *C. albicans*, and *Blastomyces dermatitidis* (6). Cross-resistance with nystatin but not pimaricin (17). Active *in vivo* against experimental *C. albicans*, *H. capsulatum*, *Coccidioides immitis*, *Cr. neoformans*,

and *Trichophyton mentagrophytes* infections of mice. Absorbed after oral administration (1, 7). Active in rabbits against *Rhizopus oryzae* infections (12), in hamsters on *H. capsulatum* (8), and in mice on *Mucor pusillus* (14) and *Paracoccidioides brasiliensis* (19). Has a protective but variable curative effect in rabbits infected with *Aspergillus fumigatus* (20).

Toxicity: LD_{50} (mice) 280 mg per kg intraperitoneally (1). Possible renal toxicity in human beings (13).

Utilization: Systemic mycoses. Coccidioidal and cryptococcal meningitis (11). Cutaneous cryptococcosis, histoplasmosis, and candidiasis (8, 13, 18). Certain cases of histoplasmosis, blastomycosis, and pulmonary coccidioidomycosis. Effective in one case each of aspergillosis, ocular candidiasis, and chromoblastomycosis (*Hormodendrum pedrosoi*) (18).

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3. Halde, C. *et al.* Antibiotics Ann. 123–127, 1956–1957.
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Angolamycin

Produced by: Streptomyces eurythermus.

Method of extraction: Culture-filtrate extracted with ethyl acetate at pH 8. Transferred into dilute aqueous acetic acid. Re-extraction with ethyl acetate after being made alkaline with Na_2CO_3. Purification by chromatography on alumina (eluent: chloroform-methanol, 16:1). Crystallized from ether. Can also be precipitated from a warmed ether solution of the crude material by seeding with the pure substance. Recrystallization from benzene on the addition of ether.

Chemical and physical properties: Basic, lipophilic substance, probably belonging to the macrolide group (2). Two crystal forms: (a) colorless crystals from benzene-ether; m.p. 133–136°C; (b) colorless needles from diisopropyl ether; m.p. 165–168°C. Ultraviolet absorption spectrum maximum at 240 mμ (log ϵ = 4.16). Infrared spectrum given in reference 1. $[\alpha]_D^{21} = -64°$ (c = 1.30 per cent in chloroform). Negative Fischback-Levine reaction for carbomycin. $C_{49\text{-}50}H_{87\text{-}91}O_{18}N$. Acid hydrolysis products include two sugars, neither of which is desosamine.

Biological activity: Active on certain gram-positive bacteria and *Endamoeba histolytica*. Not active on gram-negative bacteria, mycobacteria, or yeasts. Some activity *in vivo* on *Streptococcus pyogenes* infections in mice.

Toxicity: Mice tolerate 500 mg per kg subcutaneously.

References:

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Angustmycins

Produced by: Streptomyces hygroscopicus (1), *S. hygroscopicus* var. *angustmyceticus* (4), and *S. hygroscopicus* var. *decoyimine* (7).

Synonym: Related to psicofuranine.

Method of extraction: Broth adjusted to pH 7.6, stirred with carbon, and filtered. Carbon eluted with 80 per cent aqueous acetone at pH 2.0. Eluates are neutralized and kept in the cold over-

night. Impurities thus precipitated are discarded and the acetone solution is concentrated *in vacuo*. The concentrate is seeded and stored in the cold (2), or freeze dried (1). Freeze-dried solid taken up in hot ethanol, concentrated *in vacuo*, and purified by chromatography on alumina (benzene-ethanol, 1:1, as solvent and developer). Crystallized from benzene-ethanol (1). Component C is separated from A and B (see below) by partition chromatography on cellulose (aqueous n-butanol-1 per cent pyridine). A and B are separated by countercurrent distribution (pyridine-n-butanol-water, 1:100:100) (2). Repeated recrystallization from water (4).

Chemical and physical properties: Angustmycin is a mixture of three substances, one of which (A) is biologically active. The other two, B and C, are biologically inactive. B was identified as adenine, a structural moiety of A and C. *Angustmycin A:* Basic substance. *Hydrate:* m.p. 128–130°C, remelting at 164.5–165.5°C (decomposition) (4). Soluble in water, methanol, ethanol, pyridine, acetic acid, methyl Cellosolve, dimethylformamide, and phenol. Sparingly soluble in butanol and dioxane. Insoluble in ether, acetone, chloroform, carbon disulfide, ethyl acetate, and other organic solvents (2). Positive Molisch test. Negative Fehling and Tollen tests (4). Ultraviolet absorption spectrum maximum at 260 mμ (ϵ = 17,100) in acidic or alkaline solution or methanol (1, 2, 4). Infrared spectrum given in reference 4. $[\alpha]_D^{25}$ = +17.02° (c = 1.4 per cent in dimethylformamide) (2); pK_a = 9.8. $C_{11}H_{13}O_4N_5 \cdot H_2O$. C = 44.4%; H = 5.09%; N = 23.42%. Structural formula of angustmycin A (6-amino-9 (L-1,-2-fucopyranoseenyl)purine) (4) given in Chapter 6. *Tetraacetate:* needles; m.p. 187–188°C (4). *Benzoate:* m.p. 115–116°C (3). *Hemimethanolate:* needles; m.p. 172–174°C. Acid hydrolysis products include adenines and "angustose" or L-2-keto-fucopyranose. Angustose crystallizes as needles; m.p. 115–116°C. $[\alpha]_D^{20}$ = +18° (c = 1 per cent in ethanol), $C_6H_{10}O_5$. Rf (butanol-acetic acid-water, 4:1:5) = 0.40 (3, 4, 6). *Angustmycin C:* needles; m.p. 202–204°C (decomposition). $[\alpha]_D^{19}$ = −71.1° (c = 1.8 per cent in pyridine). Ultraviolet absorption spectrum maximum (water) at 260 mμ ($E_{1cm}^{1\%}$ 510). Optically inactive. Infrared spectrum given in reference 5. Positive Molisch test. Negative ninhydrin reaction (2, 5). $C_{11}H_{15}O_5N_5$. Structural formula of angustmycin C (6-amino-9 (β-*p*-psicofuranosyl)purine) (5) given in Chapter 6. An antibiotic, Antibiotic U 9586, said to have the same structure as angustmycin C (inactive) was reported active on bacteria and tumors *in vivo* (7).

Chemical and physical properties of Antibiotic U 9586: 6-Amino-9-D-psicofuranosylpurine. Needles; m.p. 212–214° (decomposition). Ultraviolet absorption spectrum maxima at 259 mμ ($E_{1cm}^{1\%}$ 508) in 0.01 *N* H_2SO_4 and 261 mμ ($E_{1cm}^{1\%}$ 530) in 0.01 *N* NaOH. Positive ammoniacal silver nitrate and Jordon-Pryde tests (ketohexoses) and Benedict test (after hydrolysis). Negative Bial, ninhydrin, and Benedict (before hydrolysis) tests. $[\alpha]_D^{25}$ = −537° (c = 1 per cent in dimethyl sulfoxide). $C_{11}H_{15}N_5O_5$: C = 44.25%; H = 5.10%; N = 23.74%; O = 27.02%. Acid hydrolysis products give adenine and D-psicose. Structural formula given in Chapter 6.

Biological activity: Angustmycin A inhibits *Mycobacterium* 607 and *M. phlei* at 25 μg per ml. Inactive against virulent *M. tuberculosis* H37Rv at 100 μg per ml. No activity on other bacteria or fungi (1).

Toxicity: Mice tolerate 2.5 gm per kg intraperitoneally.

References:

1. Yüntsen, H. *et al.* J. Antibiotics (Japan) **7A:** 113–119, 1954.
2. Yüntsen, H. *et al.* J. Antibiotics (Japan) **9A:** 195–201, 1956.
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Anisomycin

Produced by: Streptomyces griseolus (3, 4), *S. roseochromogenes* (14), and a *Streptomyces* sp. (3, 4).

Synonyms: Antibiotic PA 106; Flagecidin.

Method of extraction: Broth-filtrate adjusted to pH 9.0 and extracted countercurrently with methyl isobutyl ketone. Back-extracted into water at pH 2.0. This water is adjusted to pH 9.0 and extracted countercurrently with CCl_4. On concentration of this extract, anisomycin crystallizes out. Recrystallization from hot ethyl acetate or water (2). Can also be extracted from broth by diethyl ether, benzene, ethyl or butyl acetate, butanol, or chloroform. May also be recovered and purified by adsorption on charcoal,

treatment with ion exchange resins, and chromatography on alumina. May be precipitated from a concentrated chloroform-extract by addition of cyclohexane (3).

Chemical and physical properties: Basic substance. Long white needles; m.p. 140–141°C. Can be distilled *in vacuo* at a few degrees above its melting point. Soluble in lower alcohols, esters, ketones, chloroform, and dilute aqueous acids. Moderately soluble in water. Low solubility in ether, carbon tetrachloride, and hydrocarbons (3, 4). λ_{max}^{EtOH} 224 mμ (ε = 10,800), 277 mμ (ε = 1800), and 283 mμ (ε = 1600). Infrared data given in reference 4. $[\alpha]_D^{23} = -30°$ (c = 1 per cent methanol). Aqueous solutions lose potency slowly at acid pH, more rapidly at alkaline pH. Powder is stable $C_{14}H_{19}NO_4 \cdot C = 63.51\%$; H = 7.21%; N = 5.22%. Salts are very soluble in water (3, 4).

Biological activity: Active on protozoa (1), some fungi, *Candida stellatoides, C. albicans,* and *Saccharomyces cerevisiae.* Slightly active on gram-positive and gram-negative bacteria. Active *in vitro* and *in vivo* (mice) on *Trichomonas foetus* and *T. vaginalis* (3, 4, 7, 8). Some activity on *Endamoeba histolytica in vitro* (1) and in guinea pigs (5). Some activity on *Nosema* disease in bees (9). Active on powdery mildew of beans (*Erysiphe polygoni*) (11), of roses (*Sphaerotheca*) (12), and of wheat (*E. graminis* f. sp. *tritici*) (15). Also active on downy mildew of lima beans (*Phytophthora phaseoli*) (10) and broccoli (*Peronospora parasitica*) (13).

Toxicity: LD_{50} (mice) 140 mg per kg intravenously, 148 mg per kg orally, 400 mg per kg intraperitoneally. LD_{50} (rats) 72 mg per kg orally, 167 mg per kg intravenously, 345 mg per kg intraperitoneally. Causes emesis in cats and dogs. Tolerated by monkeys in daily oral doses up to 64 mg per kg over a 32-week period (7).

Utilization: Effective, in a limited clinical study, against vaginal infections with *Trichomonas vaginalis* (6).

References:

1. Lynch, J. E. *et al.* Antibiotics & Chemotherapy **4:** 844–848, 1954.
2. Sobin, B. A. and Tanner, F. W., Jr. J. Am. Chem. Soc. **76:** 4053, 1954.
3. Tanner, F. W. *et al.* U. S. Patent 2,691,618, October 12, 1954.
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Antibiotic 1-81d-1s

Produced by: Streptomyces albus.

Synonym: Has properties in common with camphomycin.

Method of extraction: From 70 to 95 per cent of the antibiotic is present in the mycelium. Mycelium separated from the broth and extracted with methanol, ethyl acetate, n-butanol, diethyl ether, benzene, chloroform, or methyl isobutyl ketone. Extract concentrated by distillation under reduced pressure. Residual aqueous slurry extracted with diethyl ether. Extract concentrated, then treated with n-pentane. Residue which precipitates is then dissolved in hot ethyl acetate, from which, after addition of hexane and cooling, the crude antibiotic precipitates. Recrystallized from ethyl acetate-aliphatic hydrocarbons, dioxane-water, ethanol-water, or chloroform-benzene mixtures.

Chemical and physical properties: Small colorless needles; m.p. 140–141°C. Soluble in lower alcohols, chloroform, ethyl acetate, dioxane, ether, and acetone. Slightly soluble in benzene. Insoluble in water, aliphatic hydrocarbons, cold 5 per cent NaOH, and cold 5 per cent HCl. $[\alpha]_D^{22} = -29.8°$ (c = 1.14 per cent in acetone). Ultraviolet absorption spectrum maxima at 244 and 284 mμ (aqueous, acidic, or dry methanol). Addition of alkali shifts the 284 mμ peak to 274 mμ. Infrared absorption spectrum given in reference 1. Heating with 5 per cent NaOH causes a gas of basic pH to be given off. Red in concentrated H_2SO_4 and lavender in cold 85 per cent H_3PO_4. Positive $KMnO_4$ test. $C_{38}H_{63\text{-}65}O_{12}N$: C = 63.10%; H = 8.87%; O = 26.48%; N = 1.92%.

Biological activity: Active on *D. pneumoniae* and *Sarcina lutea* at 6.3 μg per ml, *Streptococcus pyo-*

genes at 12.5 μg per ml, and *Staph. aureus, Streptococcus agalactiae,* and *B. anthracis* at 25 μg per ml. Inactive on other bacteria. Very active on fungi and yeasts. Complete control of tomato early blight (*Alternaria solani*) by spraying at 80 ppm. Active *in vivo* but not *in vitro* on influenza PR 8 virus in chick embryos.

Reference: 1. Herrmann, E. C. *et al.* U. S. Patent 2,805,185, September 3, 1957.

Antibiotic 10 CM

Produced by: Streptomyces sp. resembling *S. albus.*

Method of extraction: Broth adjusted to pH 2.0, autoclaved, filtered, and treated with activated carbon. Filtrate adjusted to pH 8.0. Adsorption on activated carbon and elution with acidic ethanol. Eluate evaporated *in vacuo.* Purified by chromatography on a 1:1 mixture of Celite and activated carbon. Development with ethanol.

Chemical and physical properties: Soluble in butanol; insoluble in ether. Reineckate soluble in acetone; hydrochloride much less so. Ultraviolet absorption spectrum maximum (water) at 252 mμ, with a shoulder at 260 to 280 mμ, and an inflection at 312 mμ. Stable to autoclaving at pH 2.5 for 15 minutes; less stable at neutral and alkaline pH. Distinguished from amicetin, carbomycin, erythromycin, griseomycin, methymycin, and proactinomycin by paper chromatography. Rf = 0.12 (isobutylcarbinol-n-nonanol-CCl_4-propionic acid, 50:50:25:2).

Biological activity: Active on gram-positive bacteria; less so on gram-negative bacteria. No activity on fungi or clostridia. *B. subtilis,* 1 to >100 μg per ml; *Staph. aureus,* 1 to 2 μg per ml; *Sarcina lutea,* 0.1 to 0.5 μg per ml; *Neisseria catarrhalis,* 2 μg per ml; *Sal. schottmuelleri,* 50 μg per ml; *E. coli,* 25 to >100 μg per ml; *Mycobacterium* spp., 50 μg per ml. Activity antagonized by cysteine, sodium thioglycolate, hydrazine, and hydroxylamine.

Toxicity: LD_{50} (crude powder) 500 to 700 mg per kg intravenously.

Reference: 1. Sokolski, W. T. Thesis, Purdue University, 1955.

Antibiotic 26/1

Produced by: Streptomyces globisporus.

Method of extraction: Extraction of the broth and/or the mycelium with isobutanol at pH 7 to 8. Tenfold concentration of the extract under reduced pressure at 35 to 40°C. Standing 12 to 15 hours at 4°C permits the formation of a precipitate. Further purification on anion exchanger AV-16.

Chemical and physical properties: Heptaene, weakly acidic, yellow, amorphous powder, crystallized with difficulty. Poorly soluble in water below pH 7.0. Soluble in dimethylformamide and 80 per cent diethylene glycol. Sparingly soluble in methanol, ethanol, butanol, and acetone. Insoluble in chloroform, benzene, toluene, petroleum ether, ethyl acetate, and ethyl ether. Biological activity lost rapidly at pH 4 to 5. Powder can be kept for 6 months in the refrigerator without drop in activity. Alcoholic solutions give a violet color with concentrated sulfuric acid. Negative biuret, xanthoproteic, and ninhydrin reactions. Ethanolic solutions have light absorption maxima at 359, 380, and 404 mμ. Paper chromatography (n-butanol-acetic acid-water, 20:1:25) shows that the substance belongs to the candicidin-ascosin-trichomycin group of heptaenes.

Biological activity: Active against filamentous fungi and yeasts. No activity against bacteria. Against fungi, the action is not only fungistatic but also fungicidal.

Toxicity: LD_{50} (mice) 9 to 11 mg per kg intraperitoneally, 520 to 740 mg per kg, subcutaneously. The most purified preparation has an intraperitoneal LD_{50} of 35 to 60 mg per kg.

Reference: 1. Tsyganov, V. A. *et al.* Antibiotiki 4(1): 21–26, 1959.

Antibiotic 30-10

Produced by: Streptomyces sp. (1).

Method of extraction: Broth-filtrate extracted with benzene at pH 5.4. Extract concentrated to dryness *in vacuo* (1, 2).

Chemical and physical properties: Crude substance: yellow-brown. Soluble in methanol, ethanol, butanol, amyl acetate, ether, benzene, and chloroform. Scarcely soluble in petroleum ether and water. Heat-stable at mildly acid pH (2).

Biological activity: Active on certain fungi (*e.g., Alternaria solani, Botrytis bassiana, Gloeosporium nelumbii, Colletotrichum lagenarium, Fusarium lini, Gibberella zeae, Penicillium glaucum, Rhizoctonia solani,* and *Sclerotinia*). Very slight to no activity on yeasts and bacteria (1, 2).

References:

1. Nisikado, G. *et al.* Nôgaku Kenkyû 43: 63–72, 1955.
2. Nisikado, Y. *et al.* Ber. Ōhara Inst. landwirtsch. Biol. Okayama Univ. 10: 229–240, 1956.

Antibiotic 136

Produced by: Streptomyces lavendulae and an unidentified *Streptomyces* sp. (1).

Remarks: Streptothricin-like. One of the components of broth (Fraction B) is streptothricin (1, 2).

Method of extraction: Broth-filtrate treated with carbon at pH 2.5. Adsorption on Folin Decalso at pH 7.5 and elution with 10 per cent aqueous NH_4Cl. Eluate adsorbed on Darco G-60 at pH 7.4 and eluted with 0.05 *N* HCl in 50 per cent aqueous methanol. Eluates concentrated *in vacuo*, filtered, and antibiotic precipitated out on addition of acetone. Reprecipitated from methanol with acetone. Can also be adsorbed on Super Filtrol at pH 7.0 and eluted by acidic solutions (pH 1.5 to 2.0) of the sulfates or hydrochlorides of pyridine, diethylamine, or brucine (1).

Chemical and physical properties: Basic substance. White amorphous powder. *HCl salt:* Soluble in methanol and water. *Sulfate:* Precipitated from aqueous solutions with methanol. Precipitated by Ag^+, flavianic and picrolonic acids. Most stable to heating at pH 2.0 (1). Broth contains five components; purified substance contains at least three, including streptothricin (2).

Biological activity: Active on gram-positive and gram-negative bacteria, mycobacteria, and fungi. Most active at alkaline pH. Glucose decreases activity on *Staph. albus* and *E. coli*. No activity *in vivo* on pneumococcal (Type I) infections in mice (1).

Toxicity: LD_{50} (mice) 0.2 mg per kg intravenously or subcutaneously. Orally, mice tolerate >94 mg per kg.

References:

1. Bohonos, N. *et al.* Arch. Biochem. **15:** 215–225, 1947.
2. Benedict, R. G. Botan. Rev. **19:** 229–320, 1953.

Antibiotic 156

Produced by: Streptomyces sp. resembling *S. lavendulae*.

Remarks: Supposed to belong to the streptothricin group, but has no antifungal activity.

Method of extraction: Extraction like that of streptomycin. Purified by alumina chromatography.

Chemical and physical properties: Basic polypeptide. *Sulfate:* Amorphous substance. Decomposes at 124°C. Soluble in water and methanol. $[\alpha]_D^{23} = +22.5$ (c = 1.09 per cent in H_2O). Positive biuret, Fehling, Benedict, and silver nitrate tests. Negative maltol, Sakaguchi, and $FeCl_3$ tests. Hydrolysis products include leucine, valine, proline, serine, and lysine. The peptide lysine-serine was also isolated. *Helianthate:* Plates; m.p. 280–281°C (decomposition).

Biological activity: Active on gram-positive and gram-negative bacteria and mycobacteria. No activity on fungi or viruses.

Toxicity: LD_{50} 100 mg per kg (no route given).

Reference: 1. Kawamata, J. and Fujimoto, Y. J. Antibiotics (Japan) **7B:** 192, 1954.

Antibiotic 446

Produced by: Nocardia mesenterica.

Synonym: Has properties in common with leucomycin.

Method of extraction: See mesenterin.

Chemical and physical properties: Basic substance: white; m.p. 81–87°C. Ultraviolet light absorption maxima at 230 to 231 mμ ($E_{1cm}^{1\%}$ 369) and at 280 mμ ($E_{1cm}^{1\%}$ 13.2) in ethanolic solutions. $[\alpha]_D^{22} = 82°$ (c = 0.5 per cent in ethanol). C = 60.47%; H = 7.99%; N = 2.02%. Negative Fehling reaction. Brown color upon addition of concentrated sulfuric acid. A 90 per cent loss of activity upon heating at 100°C for 30 minutes at pH 2.0. No loss at pH 4.0 to 8.0.

Biological activity: Active *in vitro* against gram-positive bacteria. No activity against gram-negative bacteria.

Toxicity: Low.

Reference: 1. Ueda, M. and Umezawa, H. J. Antibiotics (Japan) **8A:** 164–167, 1955.

Antibiotic 587/13

Produced by: An actinomycete closely related to *Streptomyces lavendulae*.

Method of extraction: Adsorption on carbon at pH 8; elution with methanol at pH 1.5 to 2.0 or with 20 per cent aqueous acetone. Precipitation with acetone after concentration of eluate. Further purification by formation of a picrate which is transformed into a hydrochloride.

Chemical and physical properties: Basic substance. Crude preparations of the hydrochloride are hygroscopic, white powders. Soluble in water, methanol, and ethanol; insoluble in ether, acetone, chloroform, benzene, ethyl acetate, and butanol. Very stable. Positive Pauly reaction; negative maltol, Sakaguchi, ninhydrin, biuret, and Molisch tests. Decolorization of permanganate solutions and bromine water.

Biological activity: Active against gram-positive and gram-negative bacteria. Active against fungi.

mainly strains of *Candida*. Effective in the treatment of experimental candidiasis.

Toxicity: Nephrotoxic to animals.

Reference: 1. Trakhtenberg, D. M. *et al.* Antibiotiki 4(2): 9–13, 1959.

Antibiotic 721

Produced by: Streptomyces sp.

Method of extraction: Isolation procedure like that for streptothricin. Purified by chromatography on alumina with methanol as solvent, and by countercurrent distribution (n-butanol-water).

Chemical and physical properties: Amber-reddish substance. Very soluble in water and methanol; soluble in ethanol and in acetone, giving a dark brown color like $FeCl_3$. On acidification, the brown-colored methanolic solution changes to straw-yellow, then to dark salmon after a few moments. On adjusting to alkaline pH the color becomes brilliant yellow. Positive Molisch test. Negative Schiff, Benedict, Millon, and Sakaguchi tests. Negative Grove-Randell tests for carbomycin and erythromycin.

Biological activity: Active on gram-positive bacteria. Inactive on mycobacteria, *Nocardia*, gram-negative bacteria (except *Neisseria* and *Brucella*), and yeasts.

Toxicity: Mice tolerate 500 mg per kg subcutaneously.

Reference: 1. Albuquerque, M. M. *et al.* Rev. inst. antibioticos 1: 89–94, 1958.

Antibiotic 1212

Produced by: Strains of blue-violet *Streptomyces*.

Synonym: Antibiotic 452–7.

Chemical and physical properties: Red-violet substance, insoluble in water, soluble in alcohol.

Biological activity: Active against gram-positive bacteria including *Staph. aureus*. Inactive *in vitro* and *in vivo* against mouse encephalomyelitis virus (Theiler's virus), experimental poliomyelitis in cotton rats, and herpes virus (Min strain). In chick embryos, marked inhibitory effect upon types A, A-1, and B influenza virus. No virucidal effect on influenza virus.

Toxicity: Mice tolerate up to 0.1 mg subcutaneously and 0.001 mg intracerebrally (doses given per 8 to 10 gm mouse).

Reference: 1. Germanova, K. I. Voprosy Virusol. 4(1): 71–76, 1959.

Antibiotic 1943

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate passed through a carboxylic-type cation exchange resin (Na^+ form). Elution with 6 per cent HCl. Most activity in the fraction at neutrality (I). Acid fractions are neutralized, filtered, and added to I. Clarified with Norit at acid pH. Filtrate neutralized and retreated with Norit. Elution with 80 per cent aqueous methanol. Methanol distilled to dryness. Residue dissolved in water and freeze dried.

Chemical and physical properties: Crystalline. Positive Sakaguchi, Molisch, and ninhydrin tests. Negative maltol and biuret tests.

Biological activity: Active on *Staph. aureus*, *B. subtilis*, *Pr. vulgaris*, and *Sal. typhosa* at 0.1 μg per ml; *K. pneumoniae*, *E. coli*, and *A. aerogenes* at 1.0 μg per ml; and *B. cereus* and *Ps. aeruginosa* at 10 μg per ml. Very weak activity on mycobacteria. Active *in vivo* on *D. pneumoniae*, *Streptococcus hemolyticus*, and *K. pneumoniae*. Active on *Sal. typhimurium* infections in mice.

Toxicity: Ototoxic to cats.

Reference: 1. Murray, F. J. *et al.* Antibiotics & Chemotherapy 7: 345–348, 1957.

Antibiotic 2814K

Produced by: Streptomyces sp. belonging to the *S. reticuli* group. This organism also produces netropsin and a pentaene antifungal antibiotic.

Synonym: Probably identical to mycolutein.

Method of extraction: Mycelium extracted with butanol. Crystallized from dimethylformamide-water or chloroform-isopropanol.

Chemical and physical properties: Long yellow-green needles or irregular yellow plates; m.p. 154–156°C. Very soluble in alcohols, ketones, esters, ether, and benzene. Soluble in chloroform, pyridine, and dimethylformamide. Poorly soluble or insoluble in petroleum ether and water. Gives an olive-green color in concentrated H_2SO_4 that rapidly changes to red-brown. Ultraviolet absorption spectrum maxima at 255 and 345 mμ. $[\alpha]_D^{24} = +44°$ (c = 0.75 per cent in chloroform). C = 66.52%; H = 6.09%; N = 3.52%.

Biological activity: Weakly fungistatic. Inhibits *P. notatum*, *P. glaucum*, and *A. niger* at >6 μg per ml.

Toxicity: Very toxic. Mice tolerate 1 mg per kg subcutaneously, but 2 mg per kg is lethal.

Reference: 1. Thrum, H. Naturwissenschaften 46: 87, 1959.

Antibiotic 6270

Produced by: Streptomyces (*Actinomyces*) *flavochromogenes* (1).

Synonym: Brazhnikova states that this anti-

biotic differs from echinomycin, but is similar to the echinomycin-like antibiotic of Berger. It may also be related to actinoleukin (1).

Method of extraction: Isolated from mycelium by extraction with acetone. Extract evaporated off and residue extracted with chloroform. Chloroform concentrated. Precipitated from concentrate by addition of petroleum ether. Chromatographed on alumina from benzene. Crystallized from acetonitrile (1).

Chemical and physical properties: Crystalline; m.p. 210–215°C. Ultraviolet absorption spectrum maximum at 320 mμ, minimum at 285 mμ. $C_{29}H_{37}O_{6\text{-}7}N_6S$; C = 65.19% (*sic*, probably 56.19%); H = 6.47%; N = 13.66%; S = 5.56% Acid hydrolysis products include serine, alanine, and dimethylleucine, the first two in equimolar quantities, not exceeding 33 per cent. Boiling with 3 per cent NaOH for 2 hours yields 1.3 moles of NH_3 (1).

Biological activity: Active at maximal tolerated levels on Crocker sarcoma and lymphosarcoma and Ehrlich's carcinoma in mice, and on sarcoma 45 (rats) (2).

Toxicity: At low doses, causes blood abnormalities and changes in the size of the spleen (2).

References:

1. Brazhnikova, M. G. Abstr. Communs. Symposium on Antibiotics. Prague, 1959, pp. 140–141.
2. Shorin, V. A. Abstr. Communs. Symposium on Antibiotics. Prague, 1959, pp. 185–186.
3. Rossolemo, O. K. *et al.* Antibiotiki 4(6): 54–59, 1959.

Antibiotic 7,080 R.P.

Produced by: Streptomyces sp. resembling *S. kitasatoensis*.

Method of extraction: Broth-filtrate extracted with butanol at acid pH. Solvent back-extracted with water at pH 7 to 8. Aqueous extract adjusted to acid pH and extracted with ethyl acetate.

Chemical and physical properties: Acidic substance; m.p. 200–205°C (decomposition). Soluble in alcohols, ethyl acetate, and pyridine. Salts soluble in water. $(C_6H_8O_5N_2)_n$: C = 38.3%; H = 4.45%; O = 42.4%; N = 14.7%. pK_a = 6. Ultraviolet absorption spectrum maxima at 227 mμ ($E^{1\%}_{1cm}$ 532), 271 mμ ($E^{1\%}_{1cm}$ 650), and 304 mμ ($E^{1\%}_{1cm}$ 663). Optically inactive.

Biological activity: Very slightly active on gram-positive and gram-negative bacteria. Not active on mycobacteria. Active *in vitro* and *in vivo* (mice, rats) on *Trichomonas vaginalis*. Active on *Endamoeba histolytica*.

Toxicity: LD_{50} (mice) 50 mg per kg subcutaneously, about 500 mg per kg orally.

Reference: 1. Despois, R. *et al.* Giorn. microbiol. **2:** 76–90, 1956.

Antibiotic AYF

Produced by: Streptomyces aureofaciens. Culture also produces tetracycline.

Synonyms: Probably synonymous with aureofacin and ayfactin.

Method of extraction: Mycelium slurried in n-butanol, adjusted to pH 9.0 to 10.0, and filtered. Extract washed with aqueous sodium ethylenediaminetetraacetate at pH 9.5. Concentration of butanol-extract precipitates antibiotic. May also be extracted from the washed concentrated butanol with water at pH 11.5 to 12.0. Acidification of aqueous phase to pH 5.0 precipitates the antibiotic. Purified by washing with methyl isobutyl ketone-chloroform-acetone (9:9:2) and water at pH 1.5. Separated into two substances by extraction with methanolic $CaCl_2$. Fraction B goes into the $CaCl_2$ solution and precipitates out on addition of water. It is reprecipitated from the same solution, then partitioned between butanol and a 2 per cent aqueous Na ethylenediaminetetraacetate solution, adjusted to pH 9.5 with NH_4OH; concentration of the butanol phase yields Fraction B. Fraction A is crystallized from dimethylformamide or dimethylacetamide by dilution with water.

Chemical and physical properties: Weakly acidic heptaenes. *Fraction A:* Dark brown crystalline substance. Insoluble in water and common organic solvents. Moderately soluble in dimethylformamide (becoming more soluble in presence of $CaCl_2$) and dimethylacetamide (solubility also enhanced by $CaCl_2$). Soluble in pyridine and dimethyl sulfoxide. Ultraviolet absorption spectrum maxima at 344, 363, 383 ($E^{1\%}_{1cm}$ 526) and 409 mμ (dimethylacetamide). C = 62.6%; H = 7.86%; N = 2.8% (Dumas) and 2.5% (Kjeldahl). *Fraction B:* Dark yellow crystalline substance. Soluble to 700 μg per ml in water at pH 3 to 10, to >2 per cent in methanolic $CaCl_2$, dimethylformamide, pyridine, and dimethyl sulfoxide, and insoluble in common organic solvents. Ultraviolet absorption spectrum the same as Fraction A except $E^{1\%}_{1cm}$ 556 at 383 mμ. Infrared spectrum given in reference 1. C = 62.4%; H = 7.62%; N = 2.8% (Kjeldahl).

Biological activity: Active on yeasts and fungi. Active *in vivo* (mice) against *C. albicans* infections.

Toxicity: Complex: LD_{50} (mice) 3.82 mg per kg intraperitoneally, >1000 mg per kg orally.

Reference: 1. Kaplan, M. A. *et al.* Antibiotics & Chemotherapy **8:** 491–495, 1958.

Antibiotic A 6

Produced by: Streptomyces sp. resembling *S. fradiae* (1).

Method of extraction: I. Culture-broth treated with carbon at pH 2.0 and filtered. Filtrate adsorbed on carbon at pH 7.0. Elution with methanol or 20 per cent aqueous acetone at pH 2.0. Addition of acetone to eluate gives precipitate. Reprecipitated from absolute methanol with ether, or from water-methanol. Purification using an ion exchange resin (1). II. Broth-filtrate treated with carbon as in I, adjusted to pH 7.2, and chromatographed on IRC-50 (Na^+ form). Eluted with 0.5 *N* HCl. Eluate adjusted to pH 5 to 6 and concentrated to dryness *in vacuo*. Precipitated from an anhydrous methanol solution of residue on addition of ether. One component (Ab-I) purified by chromatography on alumina (eluted with 50 per cent methanol) and on IRC-50 (3).

Chemical and physical properties: Basic compound. *Complex:* Contains two components. End-absorption of ultraviolet light. Positive Sakaguchi test. Negative biuret, $FeCl_3$, maltol, Schiff, Millon, xanthoproteic, nitroprusside, and Fehling tests. *Helianthate:* m.p. 203–205°C (decomposition). *Reineckate:* d.p. 285–290°C.

Biological activity: Inhibits gram-positive and gram-negative bacteria, including *Pseudomonas pyocyaneus*. Active against soft rot of a variety of vegetables caused by *Bacillus carotovorus* (2).

Toxicity: Mice tolerate 320 mg per kg intraperitoneally (3).

References:

1. Tatsumi, C. and Miyaura, D. J. Fermentation Technol. **32:** 1–7, 1954.
2. Tatsumi, C. and Miyaura, D. J. Fermentation Technol. **32:** 364–366, 1954.
3. Miyaura, J. and Tatsumi, C. J. Fermentation Technol. **33:** 533–535, 1955.

Antibiotic A 20

Produced by: Streptomyces sp.

Method of extraction: Adsorbed from culture-filtrate on carbon (Kerozite) at pH 8.2. Eluted with acetone containing 2 per cent concentrated HCl. The eluate should be about neutral after this operation; otherwise it is difficult to separate the active substance. Eluate evaporated to dryness under reduced pressure. Residue taken up in methanol, filtered to remove insoluble impurities, then precipitated with acetone. Precipitated as the picrate. Conversion to sulfate.

Chemical and physical properties: Basic antibiotic. *Hydrochloride:* Hygroscopic, amorphous, cream-colored powder. Soluble in absolute methanol. Slightly soluble in glacial acetic acid. Insoluble in ethanol, isopropanol, n-butanol, acetone, chloroform, benzyl alcohol, benzene, 1,4-dioxane, pyridine, and ethyl ether. Positive Sakaguchi, glucosamine, Tollen, and Fehling (weak) tests. Negative maltol and Molisch tests.

Biological activity: Active on gram-positive bacteria (0.52 to 20 μg per ml); less active on mycobacteria (15 to 60 μg per ml), gram-negative bacteria (3.9 to >100.0 μg per ml), and fungi (20 to 100 μg per ml). Not active on *Pseudomonas*.

Toxicity: Has a retarded toxic action at low doses; 50 mg per kg subcutaneously is lethal to mice after 72 hours. In rats, 60 mg per kg is lethal under the same conditions.

Reference: 1. Gonçalves de Lima, O. *et al.* Anais soc. biol. Pernambuco **13:** 3–9, 1955.

Antibiotic A 67

Produced by: Streptomyces sp. resembling *S. antibioticus*.

Method of extraction: Partially extracted by n-butanol. Adsorption on activated carbon and elution with 80 per cent methanol.

Chemical and physical properties: Neutral substance. Most stable at pH 7.2 to 7.5. Destroyed by boiling for 1 hour at pH 7.2. Soluble in water, methanol, ethanol, and acetone. Unstable in nonsterile sand.

Biological activity: Active on fungi, such as certain *Pythium* spp., *Metarrhizium glutinosum*, and *Aspergillus clavatus*. Less active on other fungi, such as *Sclerotinia fructicola* and *Alternaria solani*. Very slightly active on certain bacteria and yeasts. No activity on *Rhizobium* spp.

Reference: 1. Gregory, K. F. *et al.* Am. J. Botany **39:** 405–415, 1952.

Antibiotic A 116

Produced by: Streptomyces sp.

Method of extraction: Adsorbed from broth-filtrate on carbon (Kerozite) at pH 8.2. Eluted with acidic methanol. Eluate concentrated to a syrup, filtered to remove solids, and treated with acetone to precipitate other impurities. Supernatant evaporated to dryness. Residue taken up in methanol and inactive residue centrifuged off. Precipitated from methanol with acetone and ligroin. Operation repeated. Reprecipitated from methanol with acetone to give Fraction C. The supernatant from the precipitation of Fraction C evaporated to dryness. Residue taken up in isopropanol

and precipitated with acetone. Methanolic solution centrifuged to remove insoluble impurities, and supernatant evaporated to dryness to give Fraction A 10.

Chemical and physical properties: Fractions C and A 10 differ only in their reaction with picric acid: C forms a picrate; A 10 does not. C is a hygroscopic cream-colored substance; A 10 is an amorphous, hygroscopic, gray-colored substance. A *10 and C:* Positive Sakaguchi, Tollen, Benedict, and Molisch tests. Negative maltol, Millon, biuret, ninhydrin, glucosamine, and Schiff tests.

Biological activity: A *10:* Active on one strain of *B. subtilis* at 0.9 to 1.8 μg per ml, but inactive on a second. Active on micrococci, streptococci, and *Sarcina* at 0.9 to 9.2 μg per ml. Very slightly active (33 to 49 μg per ml) on *Neisseria catarrhalis* and *Brucella suis*. Inactive on other gram-negative bacteria, mycobacteria, and *Candida krusei*. *C:* very moderate activity on certain gram-positive bacteria; inactive on certain strains on which A 10 is active.

Toxicity: Mice tolerate 300 mg per kg subcutaneously.

Reference: 1. Gonçalves de Lima, O. *et al.* Anais soc. biol. Pernambuco **13:** 125–129, 1955.

Antibiotic Ax 18

Produced by: Streptomyces recifensis (formerly *Nocardia recifei* (2)).

Method of extraction: Precipitated from the culture-broth as the picrate. Conversion to the hydrochloride or sulfate, which are precipitated from a methanolic solution on addition of ligroin. Reprecipitated from absolute methanol. Conversion to orange II salt, then to the sulfate (1).

Chemical and physical properties: Basic substance. *Sulfate or hydrochloride:* Amorphous, gray substance, soluble in water and methanol. Insoluble in acetone, ligroin, and ethyl ether. Positive Sakaguchi, biuret, and ninhydrin tests. Negative maltol test (1).

Biological activity: Very slightly active on a strain of *Staph. aureus* (27.5 μg per ml) and on *B. anthracis* (55 μg per ml) (1).

References:

1. Gonçalves de Lima, O. *et al.* Anais soc. biol. Pernambuco **13:** 21–29, 1955.
2. Falcão de Morais, J. O. *et al.* Anais soc. biol. Pernambuco **15:** 239–253, 1957.

Antibiotic E 129

Produced by: Streptomyces ostreogriseus (1, 5). No description is given.

Synonyms: Ostreogrycin.

Certain antibiotic complexes, such as the actinomycins, have a confused nomenclature because numerous groups of scientists have worked with the complex in question, and each has introduced a different set of terms for the components. Only one scientific group has been responsible for the characterization of the E 129 complex, but they have introduced what appears to be two confusing sets of designations. In their patent publication (5), they refer to six components, A, B, C, D, E, and Z. Component A is synonymous with the previously described antibiotic PA 114A. Component Z is reportedly synonymous with PA 114B. They describe in some detail the component B, said to be a new substance similar chemically to A and Z. (This will be referred to below as "*Patent Component B.*") In some of their other publications (2, 3), the E 129 complex was said to contain three components, A, B, and G. Component A was synonymous with PA 114A and staphylomycin M_1. Component B was synonymous with PA 114B, and may be presumed to be the same as Z (above), but different from staphylomycin S. This Component B will hereafter be referred to as "*Other Component B.*" It was not known whether Component G was the same as staphylomycin M_2 because of the impurity of M_2. A comparison of the data available on Patent Component B and Component G does not make it possible to state whether they are the same. They are therefore treated separately below.

Method of extraction: Component G: Broth-filtrates extracted with ethyl acetate. Solvent removed under reduced pressure. Purification by fractional precipitation, chromatography, and countercurrent distribution (aqueous methanol-hydrocarbon solvent) (2). *Patent Component B:* Separated from crude complex by chromatography on alumina at pH 4.0 with ethylene dichloride as solvent and developer, or ethylene dichloride-petroleum ether (1:1) followed by a 3:1 mixture as developer. These fractions contain mainly Component A. Chloroform fractions which follow contain mainly B. A final methanol-ethyl acetate-water elution gives a fraction containing all the components. Petroleum ether added to the chloroform gives a precipitate of B. Further purified by countercurrent distribution with one of the following systems: ethyl acetate-water-methanol (4:2.5:1.5); or benzene-methanol-water (2:1:1), (4:1:3), or (3:1:2) (5).

Chemical and physical properties: Component G: White, homogeneous, amorphous solid. Highly soluble in moist polar solvents. Nearly insoluble in water, light petroleum, or carbon tetrachloride

In bromoform, has little ultraviolet absorption above 270 mμ. Infrared data given in reference 2. Positive $FeCl_3$ (green) reaction. $C_{35}H_{48}N_4O_9$ (2). *Patent Component B:* Soluble in lower alcohols, ketones, esters, methylene dichloride, acetic acid, dioxane, and dimethylformamide. Moderately soluble in benzene; slightly soluble in water (2.7 mg per ml) and the lower ethers. Insoluble in light petroleum (b.p. 60–80°C) and carbon tetrachloride. Ultraviolet absorption spectrum maximum at 215 mμ ($E^{1\%}_{1cm}$ 650) (ethanol). Infrared spectrum given in reference 5. $[\alpha]_D^{20} = -17.4°$ (c = 0.4 per cent in methanol). C = 63.25%; H = 7.10%; N = 8.05%; O = 21.60%. Molecular weight, 650. From these figures the formula $C_{34}H_{46}O_9N_4$ may be calculated (5). *All patent components:* Data on Rf values on paper chromatography given in reference 5.

Biological activity: Complex: Active *in vitro* on gram-positive bacteria, including *Staph. aureus*, *Sarcina lutea*, and *B. subtilis* (3). Most active at pH 5.5 to 7.5; less active at pH 8.5. Resistance to E 129 develops more slowly than to erythromycin, spiramycin, novobiocin, or vancomycin. Partial cross-resistance with erythromycin and spiramycin (1). *Patent Component B:* Active on the same organisms as the complex at 0.32 to 20 μg per ml (5). *Complex:* Active in mice on *Streptococcus hemolyticus* β infections (3). *Relationship of the components:* G mixed with equal quantities of "Other Component B" is six times as potent as A. Alone, all three are relatively inactive. *Mixtures of A and "Other Component B":* B potentiates an equal weight of A; any excess of A over B acts as an inert diluent. *Mixtures of "Other Component B" and G:* B potentiates an equal weight of G; any excess of one factor over the other acts as an inert diluent. *Mixtures of A, "Other Component B," and G* (not containing a large excess of B over G): Any B present preferentially potentiates an equal weight of G. Only when there is more B than G is any B available to potentiate A (3).

Toxicity: Complex: Nontoxic to mice at therapeutic levels (2).

Utilization: As effective as erythromycin on furunculosis (4).

References:

1. Garrod, L. P. and Waterworth, P. M. Brit. Med. J. **2:** 61–65, 1956.
2. Ball, S. *et al.* Biochem. J. **68:** 24P, 1958.
3. Bessell, C. J. *et al.* Biochem. J. **68:** 24P–25P, 1958.
4. Scott, A. and Waterworth, P. M. Brit. Med. J. **2:** 83–84, 1958.
5. Ball, S. and Hughes, I. W. British Patent 799,053, July 30, 1958.

Antibiotic E 300

Produced by: Streptomyces sp.

Method of extraction: Adsorption of active substance from broth-filtrate on Ionex-C (H^+) resin at pH 7.2. Elution with 80 per cent aqueous acetone. Eluate evaporated *in vacuo*. Aqueous residue extracted with ethyl acetate at pH 7.2. Extract evaporated to dryness *in vacuo*. Syrup dissolved in ethanol and precipitated with cold water at pH 2.0. Chromatographed on alumina from an ether solution and eluted with ether.

Chemical and physical properties: Brownish powder. Soluble in methanol, ethanol, butanol, amyl alcohol, acetone, ethyl acetate, chloroform, dichloroethylene, and ether. Insoluble in petroleum ether, benzene, and distilled water. Stable to boiling for 20 minutes at pH 2.0 to 9.0. Ultraviolet absorption maximum at 230 to 235 mμ (c = 0.1 per cent in aqueous methanol).

Biological activity: Not active on bacteria, fungi, or yeasts except *B. anthracis* (weak activity). Active in tissue culture on influenza PR 8 virus, possibly inhibiting intracellular growth (1, 2); also active on PR 8 *in ovo* but not in mice (3).

Toxicity: LD_{50} (mice) 100 mg per kg intraperitoneally.

References:

1. Higo, N. *et al.* Japan. J. Microbiol. **1:** 91–97, 1957.
2. Miyakawa, J. *et al.* Japan. J. Microbiol. **2:** 53–62, 1958.
3. Hinuma, Y. *et al.* Japan. J. Microbiol. **2:** 63–68, 1958.

Antibiotic EI_5

Produced by: Streptomyces sp. having gray spores and differing from *S. griseus*, *S. lavendulae*, and the actinorubin-producer.

Synonym: Possibly related to actinorubin (streptothricin-type substance).

Method of extraction: Adsorbed from broth-filtrate on carbon. Eluted with acid-methanol and precipitated on addition of acetone. Purified by chromatography on Decalso or on IRC-50. Eluates concentrated *in vacuo* and precipitated with acetone.

Chemical and physical properties: Acid salt: White substance. Soluble in water and acid-methanol. Insoluble in ether and acetone. Thermostable (resists boiling).

Biological activity: Similar to actinorubin. Strains of bacteria made resistant to EI_5 were also resistant to streptothricin, but the reverse was not uniformly true.

Toxicity: Similar to actinorubin; less toxic than streptothricin. Causes necrosis of the liver and renal damage at high concentrations.

Reference: 1. Weiser, R. S. *et al.* Proc. Soc. Exptl. Biol. Med. **72:** 283–287, 1949.

Antibiotic F 43

Produced by: Streptomyces sp.

Synonym: Related to actinoleukin and levomycin.

Method of extraction: Broth filtered at pH 7.0, and treated with acidic clay at pH 8.0. Elution with 80 per cent aqueous acetone. Acetone removed by evaporation; aqueous residue extracted with butyl acetate at pH 8.0. Extract concentrated to dryness *in vacuo*. Chromatographed on alumina from a butyl acetate-ether (2:1) solution. Developed with ethyl ether, followed by butyl acetate and ethyl ether (1:1), and finally butyl acetate which elutes the antibiotic. Precipitated from ethanol with petroleum ether. Recrystallized from ethanol at −10°C.

Chemical and physical properties: White platelets; m.p. 212–214°C. Soluble in methanol, ethanol, butanol, amyl alcohol, ethyl acetate, chloroform, and dichloroethylene. Insoluble in petroleum ether, benzene, ether, and distilled water. Positive Molisch (purple color) test. Negative ninhydrin, biuret, xanthoproteic, Hopkins-Cole, Fehling, Millon, and $FeCl_3$ tests. C = 54.33%; H = 5.95%; N = 14.13%. No S or halogens. Ultraviolet absorption spectrum maxima at 243 mμ ($E^{1\%}_{1cm}$ 425) and 320 to 325 mμ ($E^{1\%}_{1cm}$ 145) (c = 0.1 per cent in aqueous methanol). Infrared spectrum given in reference 1. Stable to boiling at pH 2.0 to 9.0 for 20 minutes.

Biological activity: Very active on gram-positive bacteria (<0.01 to 0.4 μg per ml). Not active on gram-negative bacteria, except *Sal. enteritidis* (1.6 mg per ml) and *Ps. aeruginosa*. Not active on most fungi and yeasts tested, except *A. niger* (6.3 μg per ml), *Sacch. cerevisiae* (6.3 μg per ml), and *Botrytis bassiana* (1.6 μg per ml). Active on PR 8 influenza virus *in vitro*, probably affecting the host cell in some way (1, 2). Also active on PR 8 *in ovo*, but not in mice (3).

Toxicity: LD_{50} (mice) 0.6 mg per kg intraperitoneally, 1.2 mg per kg subcutaneously.

References:

1. Higo, N. *et al.* Japan. J. Microbiol. **1:** 91–97, 1957.
2. Miyakawa, T. *et al.* Japan. J. Microbiol. **2:** 53–62, 1958.
3. Hinuma, Y. *et al.* Japan. J. Microbiol. **2:** 63–68, 1958.

Antibiotic F 256

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate extracted with n-butanol at pH 8.0. Extract evaporated *in vacuo* with addition of water. Aqueous residue extracted with ethyl ether at pH 8.0. Extract concentrated to dryness *in vacuo*.

Chemical and physical properties: White powder, soluble in methanol, ethanol, butanol, amyl alcohol, acetone, ethyl acetate, chloroform, dichloroethylene, and ether. Insoluble in petroleum ether, benzene, and distilled water. Stable to boiling for 20 minutes at pH 2.0 to 9.0. Ultraviolet absorption maximum at 275 mμ (c = 0.1 per cent in aqueous methanol).

Biological activity: Very active on gram-positive bacteria. Not active on gram-negative bacteria, fungi, or yeasts, except *A. niger* (3.2 μg per ml), *Sacch. cerevisiae* (12.5 μg per ml), and *Botrytis bassiana* (25 μg per ml). Active on influenza PR 8 virus in tissue culture.

Toxicity: LD_{50} (mice) 1 mg per kg intraperitoneally.

Reference: 1. Higo, N. *et al.* Japan. J. Microbiol. **1:** 91–97, 1957.

Antibiotic F 416

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate extracted with ethyl acetate. Extract concentrated to dryness *in vacuo*. Residue dissolved in ethanol and precipitated with cold water at pH 2.0. Chromatographed from ether-petroleum ether (9:1) solution on alumina. Developed with petroleum ether, ether and petroleum ether (1:1), then ether. Ether fraction evaporated to dryness, then precipitated from an ether-petroleum ether (9:1) solution at room temperature.

Chemical and physical properties: Colorless needles; m.p. 114–117°C. Soluble in methanol, ethanol, butanol, amyl alcohol, acetone, ethyl acetate, chloroform, dichloroethylene, and ether. Not soluble in petroleum ether, benzene, or distilled water. Negative Molisch, Fehling, Millon, xanthoproteic, $FeCl_3$, biuret, and Hopkins-Cole tests. Stable at pH 5.0 to 7.0 but not 2.0 or 9.0 when boiled 20 minutes. Ultraviolet absorption spectrum showed only end-absorption (c = 0.1 per cent in aqueous methanol).

Biological activity: Not active on bacteria, except *Sarcina lutea* (<0.05 μg per ml) and *Micrococcus citreus* (<0.05 μg per ml). Not active on fungi and yeasts, except *A. niger* (3.2 μg per ml), *Absidia orchitis* (6.3 μg per ml), and a *Mycotorula* sp. (12.5 μg per ml). Active in tissue culture on

influenza PR 8 virus, probably by direct inactivation of free virus particles (2).

Toxicity: LD_{50} (mice) 40 mg per kg intraperitoneally.

References:

1. Higo, N. *et al.* Japan. J. Microbiol. **1:** 91–97, 1957.
2. Miyakawa, T. *et al.* Japan. J. Microbiol. **2:** 53–62, 1958.

Antibiotic GB/229

Produced by: Streptomyces sp. possibly related to *S. roseus.*

Method of extraction: Adsorbed on Darco G-60 from broth-filtrate at pH 6.0 to 6.6. Eluted with dilute aqueous HCl (pH 3.0) and eluate lyophilized. Precipitated from an aqueous solution on addition of acetone. Purified by salt interconversion.

Chemical and physical properties: Hydrochloride: Very soluble in water. $[\alpha]_D = -32.5°$ (in water). No absorption of ultraviolet light. Infrared spectrum not very characteristic. Positive Tollen and Fehling tests; weakly positive ninhydrin and biuret tests; negative Molisch and Schiff tests. N = 13.49%; Cl = 20.4%. Stable between pH 2.5 and 8.0 and at room temperature. Rf = 0.90 (3 per cent ammonium chloride); = 0.15 (acetone-H_2O, 1:1). The reineckate and helianthate are hardly soluble in water, the picrate slightly soluble.

Biological activity: Active on gram-positive and gram-negative bacteria, mycobacteria, fungi, and yeasts at 1 to 20 μg per ml. Active on *Ps. aeruginosa* at 50 μg per ml. Cross-resistance with neomycin and streptomycin.

Toxicity: LD_{50} (rats) 20 mg per kg intravenously.

Reference: 1. Rolland, G. *et al.* Rass. med. sper. **3:** 1–6, 1956.

Antibiotic J4

Produced by: Streptomyces sp. belonging to the *S. fungicidicus* "group G."

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 4.0. Concentrated extract passed through an alumina column, mixed with water, and solvent evaporated off *in vacuo.* Yellow precipitate dissolved in ether. Ether concentrated to precipitate J4. Recrystallized from methanol and chloroform.

Chemical and physical properties: White needles; m.p. 175°C. Very soluble in alcohol and ether; fairly soluble in ethyl acetate, acetone, and chloroform; insoluble in water. No characteristic ultraviolet absorption spectrum. C = 66.79%; H = 6.48%; N = 10.33%; O = 17.40%. Negative ninhydrin, Sakaguchi, biuret, Millon, Molisch, Seliwanoff, $FeCl_3$, Tollen, and Fehling tests.

Biological activity: Active on gram-positive bacteria, including *Mycobacterium avium.*

Reference: 1. Taguchi, H. and Nakano, A. J. Fermentation Technol. **35:** 145–149, 1957.

Antibiotic K 125a

Produced by: Streptomyces sp. This organism produces at least two antibiotics.

Chemical and physical properties: Yellow powder; m.p. 194°C (decomposition). Insoluble or sparingly soluble in water, ether, ethyl acetate, and acetone. Readily soluble in alcohols. Negative Sakaguchi, Millon, biuret, and $FeCl_3$ tests. Purple color in concentrated H_2SO_4. Stable between pH 6 and 8.

Biological activity: Primarily active on gram-positive bacteria. Some gram-negative bacteria and fungi also inhibited.

Reference: 1. Okuda, T. *et al.* J. Antibiotics (Japan) **7B:** 4–6, 1954.

Antibiotic L. A. 7017

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 2.0. Extracts distilled to dryness *in vacuo* under N_2. Residue dissolved in acetone and chromatographed on alumina. Active fractions concentrated under N_2. Isopropyl ether is added to concentrate, and mixture concentrated *in vacuo* until a flocculent green-yellow precipitate forms. Purification by countercurrent distribution (phosphate buffer pH 7.3—butanol, 1:1). Addition of petroleum ether to active fractions causes activity to transfer to aqueous phase, which is extracted at pH 2.5 with ethyl acetate. Extract concentrated *in vacuo*, and antibiotic precipitated on addition of isopropyl ether.

Chemical and physical properties: Acidic. Green-yellow powder; m.p. 154–157°C (decomposition). $[\alpha]_D^{25} = -155°$ (c = 0.4 per cent in ethanol). C = 56.99%; H = 7.18%. No N, S, P, or halogens. Very soluble in most organic solvents. Insoluble in dialkyl ethers, petroleum ether, and ligroin. Fairly soluble in bicarbonate, with CO_2 evolution, and in alkaline or neutral buffers. Slightly soluble in water. Decolorizes $KMnO_4$. Does not absorb Br_2 and gives a brown color with $FeCl_3$. Negative Fehling test. pK′ = 5.0 and pK″ = 9.5. Ultraviolet absorption spectrum maxima (methanol) at 280.5 mμ ($E_{1cm}^{1\%}$ 470) and 430 mμ ($E_{1cm}^{1\%}$ 103). Infrared spectrum given in reference 1.

Biological activity: Active on gram-positive bac-

teria. Not active on gram-negative bacteria or yeasts.

Toxicity: LD_{50} (mice) 0.35 mg per kg intravenously.

Reference: 1. Sensi, P. *et al.* Antibiotics & Chemotherapy **8:** 241–244, 1958.

Antibiotic PA 86

Produced by: Streptomyces rimosus.

Method of extraction: Whole culture filtered with a filter-aid at acid pH. Filtrate extracted with butanol at pH 7.8 to 9.0. Butanol evaporated *in vacuo* with addition of water, and cooled to give a precipitate. Taken up in hot methanol, filtered, and water added. Solvent is evaporated off until a solid precipitates. May also be extracted from the broth-filtrate with benzyl or amyl alcohol. Recrystallized from methanol-water or hot 50 per cent aqueous acetone.

Chemical and physical properties: Tetraene. White needles, small plates, or rosettes; m.p. 230–235°C (decomposition). Soluble in aqueous alcohols. Low solubility in water, alcohols, ethers, and ketones. Ultraviolet absorption spectrum maxima at 279, 291, 304, and 318 mμ. Infrared spectrum given in reference 1. Optically inactive in common solvents. Unstable in acid. C = 60.30%; H = 8.30%; N = 3.41%. No S, P, or halogens.

Biological activity: Active on yeasts and filamentous fungi.

Reference: 1. British Patent 719,878, December 8, 1954.

Antibiotics PA 108, PA 133A, PA 133B, and PA 148

Produced by: Streptomyces sp.

Synonyms: Macrolide-type antibiotics related to spiramycin, narbomycin, leucomycin, borrelidin, and angolamycin.

Method of extraction: Extraction of filtered broth with ethyl acetate or methyl isobutyl ketone. Solvent evaporated to dryness *in vacuo*. Further purification by countercurrent distribution in the system benzene-cyclohexane-95 per cent ethanol-water, 5:5:8:2 by volume. Coefficient distributions in that system: PA 108 = 0.43; PA 133A = 1.50; PA 133B = 0.50; PA 148 = 0.17; carbomycin = 0.41; carbomycin B = 0.67; erythromycin = 0.24; oleandomycin = 0.25.

Chemical and physical properties: PA 108: m.p. 121–123°C. Proposed empirical formula: $C_{38}H_{63}NO_{14}$. $[\alpha]_D^{25} = -36.8°$ (c = 1 per cent in $CHCl_3$). Light-absorption maximum at 279 mμ ($E_{1cm}^{1\%}$ 289). *PA 133A:* Amorphous material melts at 87.2–88.0°C. Tentative empirical formula: $C_{25}H_{43}NO_6$. $[\alpha]_D^{25} = +39.6°$ (c = 0.5 per cent in methanol). Light-absorption maxima at 226 mμ ($E_{1cm}^{1\%}$ 183) and at 275 mμ ($E_{1cm}^{1\%}$ 9.0). *PA 133B:* m.p. 99.8–101°C. Tentative empirical formula: $C_{25}H_{45}NO_{10}$. $[\alpha]_D^{25} = +22.5°$ (c = 0.5 per cent in methanol). Light absorption maximum at 223 mμ ($E_{1cm}^{1\%}$ 184). *PA 148:* Amorphous material; melts at 115–118°C. Tentative empirical formula: $C_{38}H_{65}NO_{15}$. $[\alpha]_D^{25} = -69.3°$ (c = 0.5 per cent in methanol). Light-absorption maximum at 238 mμ ($E_{1cm}^{1\%}$ 153) with a shoulder at 280 mμ. Infrared absorption spectra and paper chromatography data given in reference 1.

Biological activity: PA 108: About as active as carbomycin against *B. subtilis* in a cylinder plate assay. *PA 133A* and *PA 133B:* About one fourth as active as carbomycin against *B. subtilis* in a cylinder plate assay.

Reference: 1. Murai, K. *et al.* Antibiotics & Chemotherapy **9:** 485–490, 1959.

Antibiotic PA 114

Produced by: Streptomyces olivaceus (2). The organism produced a number of minor components in addition to PA 114A, B, and B-3, described below (4).

Synonyms: PA 114A is identical to E 129A and staphylomycin M_1. PA 114B is the same as E 129B but differs from staphylomycin S (3).

Method of extraction: Broth-filtrate extracted with methyl isobutyl ketone or other solvents, including ether, benzene, ethyl acetate, butanol, or chloroform (1, 2). Extract concentrated and chilled with precipitation of 114A. Addition of hexane to the supernatant gives a precipitate. Precipitate taken up in methylene chloride. Impurities precipitated by addition of carbon tetrachloride and concentration. Addition of hexane to the concentrate gives 114B. 114A is purified by countercurrent distribution (benzene-methanol-water, 2:1:1). 114A crystallizes from hot n-butyl ethyl ketone or methanol-water. 114B and 114B-3 also purified by countercurrent distribution (toluene-methanol-water, 4:3:1). Further purified by chromatography on silica gel followed by redistribution countercurrently. Crystallized from methanol or toluene-hexane (1, 4).

Chemical and physical properties: Complex: Amorphous yellow powder. Soluble in lower alcohols, chloroform, acetone, dioxane, benzene, ether, and methylene chloride. Sparingly soluble in water and carbon tetrachloride. Insoluble in hexane. Infrared spectrum data given in reference 2. *PA 114A:* Neutral polypeptide. Colorless needles; m.p. 200°C (decomposition). Ultraviolet absorp-

tion spectrum maxima (methanol) at 220 to 230 mμ ($E^{1\%}_{1cm}$ 655) and 275 mμ ($E^{1\%}_{1cm}$ 200). Infrared absorption spectrum given in reference 1. $[\alpha]^{25}_{D}$ = −207° (c = 0.5 per cent in methanol). Positive $FeCl_3$ (green), neutral permanganate, Br_2 in CCl_4, Tollen, 2,4-dinitrophenylhydrazine, and copper acetate tests. Negative Fehling, Molisch, Millon, and ninhydrin tests. $C_{35}H_{42}N_4O_9$ or $C_{25}H_{31}N_3O_6$: C = 63.7%; H = 6.48%; N = 8.61%. Molecular weight, 525. *PA 114B:* Weakly acidic polypeptide. Colorless tablets (from methanol) or needles (from toluene); m.p. 265°C (decomposition). Ultraviolet absorption maxima (methanol) at 260 mμ ($E^{1\%}_{1cm}$ 217) and 305 mμ ($E^{1\%}_{1cm}$ 105). $[\alpha]^{25}_{D}$ = −59.7° (c = 0.5 per cent in methanol). Infrared absorption given in reference 1. Positive $FeCl_3$ (red), neutral permanganate, Br_2 in CCl_4 tests. Negative Tollen, 2,4-dinitrophenylhydrazine, copper acetate, Fehling, Molisch, Millon, and ninhydrin tests. $C_{52}H_{63}N_9O_{12}$: C = 62.02%; H = 6.21%; N = 12.77%. Molecular weight, 981. *PA 114B-3:* Polypeptide differing slightly in amino acid content from PA 114B. Needles; m.p. 207–208°C. $[\alpha]^{28}_{D}$ = −37.2°. Ultraviolet absorption spectrum maxima (methanol) 258 mμ ($E^{1\%}_{1cm}$ 204.5) and 305 mμ ($E^{1\%}_{1cm}$ 95). C = 62.77%; H = 6.52%; N = 12.61% (1, 2, 4).

Biological activity: PA 114A and 114B have a synergistic action. PA 114B-3 and 114A have a marked synergistic action *in vitro*. PA 114 B-3 and 114B have a similar, but less marked synergistic activity. Complex is active on gram-positive bacteria, mycobacteria, and the genera *Neisseria* and *Hemophilus*, but not against other gram-negative bacteria. Not active on fungi. Some cross-resistance with carbomycin and erythromycin, but not other commonly used antibiotics. Very slightly active (250 μg per ml) on *Endamoeba histolytica*. Resistance develops slowly and follows the penicillin pattern. *In vitro* activity not reduced by NaCl, glucose, cysteine, thioglycollate, urea, or serum. Artificial mixtures of A and B are active over a wide ratio of concentrations within the limits of 20 to 80 per cent. Active *in vivo* on infections caused by *Staph. aureus* and *Streptococcus pyogenes* (mice). Somewhat active *in ovo* and in mice on *Rickettsia akari* and the psittacosis organism. Alone, neither A nor B is active *in vivo*. Maximal protection with artificial mixtures is obtained with the following combinations: PA 114A 80 to 95 per cent; PA 114B 5 to 20 per cent (1, 2, 4). This differs from E 129 complex.

Toxicity: Mice tolerate 200 to 400 mg per kg orally and subcutaneously. Rabbits tolerate at least 100 mg per kg intramuscularly (1, 2).

References:

1. Celmer, W. D. *et al.* Antibiotics Ann. 437–452, 1955–1956.
2. Sobin, B. A. *et al.* U. S. Patent 2,787,580, April 2, 1957.
3. Ball, S. *et al.* Biochem. J. **68:** 24P, 1958.
4. Hobbs, D. C. and Celmer, W. D. Federation Proc. **18:** 246, 1959.

Antibiotic PA 128

Produced by: Streptomyces sp.

Method of extraction: Broth and mycelium extracted with n-butanol at pH 7.0 to 8.0. Clarified extracts concentrated under reduced pressure with addition of water. Purification of residue by countercurrent distribution (ligroin, b.p. 60–90°C, and 80 per cent aqueous methanol). Active fractions concentrated to remove methanol, then extracted with ether. Concentration of ether-extracts gives crude substance. Recrystallization from ether.

Chemical and physical properties: Light yellow rectangular plates; m.p. 143–144°C (decomposition). Slightly soluble in water. Soluble in lower alcohols, acetone, ethyl acetate, and chloroform. Ultraviolet absorption maxima at 245 mμ ($E^{1\%}_{1cm}$ 468) and 285 mμ ($E^{1\%}_{1cm}$ 234). Infrared spectrum given in reference 1. $[\alpha]^{25}_{D}$ = −2.0° (c = 1 per cent in methanol). Positive 2,4-dinitrophenylhydrazine, bromine water, and $KMnO_4$ tests. Negative $FeCl_3$ test. No color with aqueous NaOH or H_2SO_4.

Biological activity: Active *in vitro* on *Trichomonas vaginalis* and *Endamoeba histolytica*. Not active on gram-positive or gram-negative bacteria or fungi. No activity *in vivo*.

Toxicity: Very toxic; 25 mg per kg daily for 5 days is lethal to mice.

Utilization: Possible utilization in studies *in vitro* on protozoal metabolism.

Reference: 1. Rao, K. V. and Lynch, J. E. Antibiotics & Chemotherapy **8:** 437–440, 1958.

Antibiotic PA 132

Produced by: Streptomyces sp.

Method of extraction: Broth, acidified to pH 2.5 with dilute H_2SO_4, extracted with an organic solvent such as chloroform, ether, or methyl isobutyl ether. Extract concentrated *in vacuo*. Concentrate chromatographed on acid-washed alumina and developed with chloroform followed by 2.5 per cent methanol in CCl_4. Also purified by countercurrent distribution (toluene-methanol-water). Ether solution treated dropwise with benzylamine with stirring. Yellow precipitate triturated with ethyl acetate-ether (1:9) to yield

buff-colored benzylamine salt. A 50 per cent aqueous methanol solution of this salt is adjusted to pH 2.5 and extracted with ether; this gives free base on evaporation to dryness (1).

Chemical and physical properties: Labile, lactonic acid (I) has a biologically active, stable, crystalline monobenzylamine salt (II). It is a colorless, amorphous powder which darkens on standing. $[\alpha]_D^{25} = -161°$ (c = 1 per cent in methanol). Ultraviolet absorption spectrum has one peak at 218.5 mμ ($E_{1cm}^{1\%}$ 358). Positive permanganate and bromine tests. Negative $FeCl_3$ (yellow-green), Fehling, 2,4-dinitrophenylhydrazine, Tollen alcoholic silver nitrate, and sodium hypoiodite tests. No S, N, or halogens. $C_{16}H_{18-20}O_5$: C = 64.67%; H = 6.29%, 2 methyl groups, but no methoxyl group. pK_a = 5.3 (50 per cent aqueous ethanol). Neutral equivalent, 292. Soluble in most organic solvents. Insoluble in water. Infrared spectrum given in reference 1. II: m.p. 128–131°C. $[\alpha]_D^{25} = -130°$ (c = 1 per cent in methanol). Neutral equivalent, 373. pK_a = 8.9 (50 per cent aqueous ethanol). Infrared spectrum in reference 1. Soluble in methanol. Slightly soluble in water and ethyl acetate. Insoluble in hexane and ether (1).

Biological activity: Active on gram-positive bacteria including *Clostridium perfringens*, *Streptococcus pyogenes*, *D. pneumoniae*, *Erysipelothrix rhusiopathiae*, and *Corynebacterium diphtheriae* (0.19 to 0.78 μg per ml). Less active on *Staph. aureus* strains resistant to other antibiotics. Moderately active on some gram-negative bacteria, including *E. coli*. Not active on mycobacteria. Very active on *Trichomonas vaginalis* and *Endamoeba histolytica*. Slightly active on yeasts and saprophytic fungi; moderately active on phytopathogenic fungi. *In vivo*, toxicity is too great to give effective doses (2).

Toxicity: LD_{50} (mice) 12.5 mg per kg subcutaneously, 25 mg per kg orally (2).

Utilization: Possibly against plant disease (2).

References:

1. Koe, K. B. *et al.* Antibiotics Ann. 672–675, 1956–1957.
2. English, A. R. *et al.* Antibiotics Ann. 676–681, 1956–1957.

Antibiotic PA 147

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate extracted with methyl isobutyl ketone. Extracts concentrated *in vacuo*. Back-extracted into pH 6.8 phosphate-acetic acid buffer. Buffer adjusted to pH 2 and extracted with ethyl acetate. Extract concentrated *in vacuo*. Purified by chromatography on alumina from ethyl acetate concentrate. Forms a crystalline barium salt.

Chemical and physical properties: (3-Carboxy-2,4-pentadienal lactol.) *Ba salt:* Faintly yellow, crystalline powder. $C_{12}H_{10}O_6 \cdot BaH_2O$: C = 35.83%; H = 3.12%; Ba = 34.03%; H_2O =3.10%; CH_3-C = 2.24%; CH_3CO = 3.08%. Optically inactive. Ultraviolet absorption spectrum maximum at 272 mμ (ε = 2200). *Free acid:* $C_6H_6O_3$. Ultraviolet maximum 272 mμ (ε = 17200). Reacts with benzylamine to give a purple-red solid. Structural formula of free acid given in Chapter 6. *Hydrogenation product:* Colorless oil; b.p. 120–125°C. Optically inactive.

Biological activity: Ba salt has weak activity against *Pasteurella multocida* (62.5 μg per ml). Even less active on other bacteria (mainly gram-positive bacteria and *Hemophilis*). Not active on yeasts (2).

Toxicity: Free acid: LD_{50} (mice) 315 mg per kg (route unknown) (2).

References:

1. Els, H. *et al.* J. Am. Chem. Soc. **80:** 878–880, 1958.
2. Sobin, B. A. Personal communication, 1958.

Antibiotic PA 150

Produced by: Streptomyces sp. (2).

Method of extraction: Mycelium extracted with water-saturated butanol. Extract concentrated *in vacuo* until antibiotic precipitates. Purification by fractional precipitation from a methanolic calcium chloride solution with water, followed by conversion to a crystalline salt (*e.g.*, monosodium or triethylamine sulfate double salt). Precipitation of the crystalline amphoteric form from an aqueous alcohol solution of the salt by neutralization with dilute acid or alkali and cooling (2).

Chemical and physical properties: Amphoteric heptaene. Yellow substance. Antibiotic and salts show gradual darkening and decomposition up to 260°C. Slightly soluble in pyridine and dimethylformamide; less soluble in methanol, ethanol, propanol, butanol, and dioxane. Insoluble in water, acetone, methyl isobutyl ketone, ethyl acetate, chloroform, benzene, and methyl cyclohexane. Solubility in alcohols enhanced by water. Triethylamine sulfate and sulfate salts soluble as above. HCl and monosodium salts more soluble in polar solvents. Ultraviolet absorption spectrum maxima at 340, 358, 377 ($E_{1cm}^{1\%}$ 1033), and 397 mμ (80 per cent aqueous methanol). $[\alpha]_D^{25} = +294°$ (pyridine) or −34° (dimethylformamide-0.1 *N* HCl). Positive Fehling and 2,4-dinitrophenylhydrazine tests. Weakly positive ninhydrin test. Blue color in concentrated H_2SO_4. Infrared spec-

trum given in reference 2. $C_{54}H_{82}N_2O_{18}$: C = 62.03%; H = 7.83%; N = 2.73%; C-CH_3 = 5.48%. No methoxyl or acetyl groups. Light- and heat-labile. Most stable at neutrality. *Sodium salt:* $[\alpha]_D^{25}$ = −2590° (water). *Hydrochloride:* $[\alpha]_D^{25}$ = +140° (c = 0.4 per cent in dimethylformamide).

Biological activity: Active on yeasts and filamentous fungi (1).

Toxicity: LD_{50} (mice) 2.25 mg per kg subcutaneously, and 14 mg per kg orally (1).

References:

1. English, A. R. and McBride, T. J. Antibiotics Ann. 893–896, 1957–1958.
2. Koe, B. K. *et al.* Antibiotics Ann. 897–905, 1957–1958.

Antibiotic PA 153

Produced by: Streptomyces sp. (2).

Method of extraction: Same as antibiotic PA 150 (2).

Chemical and physical properties: Amphoteric pentaene. Colorless needles exhibiting strong gray-green fluorescence under ultraviolet light. Solubilities and melting point same as PA 150. Ultraviolet absorption spectrum maxima at 303, 317, 332, and 349 mμ ($E_{1cm}^{1\%}$ 1445) (80 per cent aqueous methanol). Infrared spectrum given in reference 2. $[\alpha]_D^{25}$ = +398° (pyridine) or +353° (dimethylformamide-0.1 *N* HCl). Positive ninhydrin, 2,4-dinitrophenylhydrazine, and Fehling tests. Violet color in concentrated H_2SO_4 . $C_{37}H_{61}NO_{14}$: C = 59.94%; H = 8.29%; N = 1.88%; C-CH_3 = 6.01%. No methoxyl or acetyl groups. Heat- and light-labile. Most stable at pH 7 to 10. *Sodium salt:* $[\alpha]_D^{25}$ = +205° (methanol). *Hydrochloride:* $[\alpha]_D^{25}$ = +283° (c = 0.4 per cent in dimethylformamide).

Biological activity: Moderately active on yeasts and filamentous fungi (1).

Toxicity: LD_{50} (mice) >200 mg per kg subcutaneously and >400 mg per kg orally (1).

References:

1. English, A. R. and McBride, T. J. Antibiotics Ann. 893–896, 1957–1958.
2. Koe, B. K. *et al.* Antibiotics Ann. 897–905, 1957–1958.

Antibiotic PA 155A

Produced by: Streptomyces albus (2).

Method of extraction: Extraction of broth with ethyl acetate. Solvent concentrated and treated with an excess of heptane to precipitate the crude antibiotic. Countercurrent distribution in the system benzene-methanol-water (2:1:1). Active fractions chromatographed on acid-washed alumina in ethyl acetate. Elution with 2 to 5 per cent methanol in ethyl acetate. Concentration of the solvent gives a colorless crystalline solid (1).

Chemical and physical properties: Very weak base. Suggested empirical formula: $C_{14}H_{15}O_2N_3$. Colorless rectangular prisms; m.p. 209–210°C. $[\alpha]_D^{25}$ = −214° (c = 2 per cent in methanol). Maxima of light absorption at 218, 273, 281, and 288 mμ. Spectrum similar to that of tryptophan. Slightly soluble in water, benzene, and ether. Moderately soluble in lower alcohols and acetone. Negative $FeCl_3$, ninhydrin, and 2,4-dinitrophenylhydrazine reactions. Deep blue color with Ehrlich reagent in strong alkali. Bromine water decolorized. Positive neutral permanganate reaction. Infrared absorption spectrum given in reference 1. Crystalline picrate contains 2 moles of picric acid to 1 mole of PA 155A. Stable for 1 hour at 100°C in the pH range 2.0 to 8.0. Not stable at an alkaline pH (1).

Biological activity: Active against gram-positive bacteria.

References:

1. Rao, K. V. Antibiotics & Chemotherapy **10:** 312–315, 1960.
2. Marsh, W. S. *et al.* Antibiotics & Chemotherapy **10:** 316–319, 1960.

Antibiotic PA 166

Produced by: Streptomyces sp. (2).

Method of extraction: Same as antibiotic PA 150 (2).

Chemical and physical properties: Amphoteric tetraene. Colorless needles. Same solubilities and melting point as PA 150. Ultraviolet absorption spectrum maxima at 291, 304 ($E_{1cm}^{1\%}$ 1098), and 319 mμ (80 per cent aqueous methanol). Infrared spectrum given in reference 2. Same test reactions as PA 153. $C_{35}H_{53}NO_{14}$: C = 59.59%; H = 7.66%; N = 2.00%; C-CH_3 = 6.77%. No methoxyl or acetyl groups. Light- and heat-labile. Most stable from pH 7.0 to 10.0. *Sodium salt:* $[\alpha]_D^{25}$ = +194° (methanol). *Hydrochloride:* $[\alpha]_D^{25}$ = +239° (dimethylformamide) (2).

Biological activity: Active on yeasts and filamentous fungi. No activity in mice on *C. albicans* (1).

Toxicity: LD_{50} (mice) >800 mg per kg subcutaneously, >1000 mg per kg orally. Nonirritating topically in rabbits (1).

References:

1. English, A. R. and McBride, T. J. Antibiotics Ann. 893–896, 1957–1958.
2. Koe, B. K. *et al.* Antibiotics Ann. 817–905, 1957–1958.

Antibiotic SAX 10

Produced by: Streptomyces aureus.

Synonyms: Resembles antitumor antibiotic 289. The authors (1) state that it is related to luteomycin, but differs in ultraviolet absorption, solubility, and biological activity.

Method of extraction: Agar plate cultures extracted with acetone or acidic water. Extracted from the water with ethyl acetate, butanol, chloroform, or benzene. Recrystallized from benzene-petroleum ether.

Chemical and physical properties: Brown-orange needles. Blackens at 150–160°C; no melting point up to 250°C. Soluble in chloroform, acetone, methanol, and benzene. Slightly soluble in water and petroleum ether. Ultraviolet absorption spectrum maxima at 215, 255, and 430 mμ in 0.1 *N* HCl. Infrared absorption spectrum given in reference 1. Negative ninhydrin, Molisch, xanthoproteic, and Tollen tests. Purple color in 10 per cent sodium carbonate solution; deep orange in concentrated H_2SO_4. Alcoholic solution turns purple on addition of magnesium acetate. Data on paper chromatographic behavior given in reference 1. $C_{29}H_{33}NO_9 \pm CH_2$: C = 64.78%; H = 6.43%; N = 2.51%. No halogen or S. *Hydrochloride:* Soluble in methanol, acetone, and water. Sparingly soluble in ether and benzene.

Biological activity: Active on gram-positive bacteria. Inactive on mycobacteria, gram-negative bacteria, and fungi.

Reference: 1. Kinoshita, S. and Nakayama, K. J. Antibiotics (Japan) **9B:** 319–323, 1956.

Antibiotic SKCC 1377

Produced by: Streptomyces sp.

Method of extraction: Extraction of culture-filtrate with benzene. Concentration *in vacuo*, back-extraction with 0.05 *N* HCl, lyophilization.

Chemical and physical properties: Red-brown powder. Soluble in water, ethanol, and acetone. Insoluble in ether or benzene. In aqueous solution, yellow at acid or neutral reaction and purple at alkaline pH. Unstable at alkaline reaction. Is stable for 10 minutes at 100°C, pH 3.5. Picrate crystals melt with decomposition at 165–168°C. Maximal light absorption of the picrate at 245 and 255 mμ.

Biological activity: Active against gram-positive bacteria, but not active against *E. coli* and fungi.

Toxicity: LD_{50} (mice) 5 mg per kg intraperitoneally.

Reference: 1. Reilly, H. C. Bacteriol. Proc. 26, 1952.

Antibiotic X 206

Produced by: Streptomyces sp.

Method of extraction: Can be extracted from both the mycelium and the broth-filtrate. Broth extraction: extraction with butyl acetate, concentration *in vacuo*, back-extraction with phosphate buffer (pH 8.9). Mycelium extraction: extraction with ethanol and methanol, concentration *in vacuo*, extraction with butyl acetate, back-extraction with phosphate buffer (pH 8.9). Further purification of both extracts by chromatography on alumina.

Chemical and physical properties: Colorless organic acid; m.p. 126–128°C. $[\alpha]_D^{29} = +15.0°$ in methanol. No characteristic absorption in the ultraviolet. Unstable at acid reaction or in alkaline solutions. Soluble in alcohols, esters, acetone, ether, and petroleum ether. Insoluble in water and alkali. $C_{46-47}H_{80-82}O_{13}$.

Biological activity: Active *in vitro* against gram-positive bacteria and mycobacteria. Not active *in vivo* against bacterial and protozoan infections.

Toxicity: LD_{50} (mice) 11 mg per kg subcutaneously.

Reference: 1. Berger, J. *et al.* J. Am. Chem. Soc. **73:** 5295–5298, 1951.

Antibiotic X 340

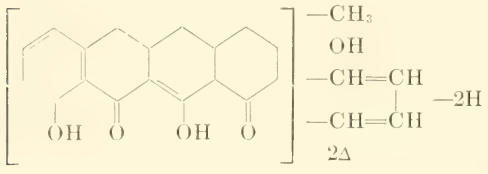
Produced by: Streptomyces sp.

Synonyms: May be related to resistomycin. Nucleus similar to that of the tetracyclines.

Method of extraction: Broth-filtrate extracted with ether at pH 4.5. Evaporation of ether. Crystallization from ethanol, methanol, or dimethylformamide. Mycelial mat extracted in a Soxhlet apparatus with petroleum ether to remove inactive impurities, then with ether followed by chloroform. Also extracted from the dried, powdered mycelium with butanol. Butanol concentrated *in vacuo*, filtered to remove inactive precipitates, then evaporated to dryness. Residue dissolved in 0.1 *N* NaOH, filtered, then acidified to precipitate the antibiotic.

Chemical and physical properties: Bright yellow needles; m.p. 330–331°C (decomposition). Soluble in ether, chloroform, carbon tetrachloride, ethyl acetate, n-butanol, ethanol, methanol, dimethylformamide, and pyridine. Sparingly soluble in water, acetone, benzene, and petroleum ether. Alkaline solution is dark red. Positive $FeCl_3$ test. Negative 2,4-dinitrophenylhydrazine reaction. No N, halogen, or S. $C_{23}H_{26}O_6$: C = 70.31%; H = 5.01%; C-CH_3 = 4.68%. Molecular weight (Rast) 404 ± 40. $pK_a = 7.66$. Infrared spectrum given in reference 1. Ultraviolet absorption spec-

trum maxima: *in ethanol:* 218 mμ (log ε 4.66), 289 mμ (log ε 4.60), 370 mμ (log ε 4.06) with shoulders at 273 mμ (log ε 4.39) and 305 mμ (log ε 4.48); *in 0.1 N ethanolic HCl:* 269 mμ (log ε 4.46), 292 mμ (log ε 4.40), 321 mμ (log ε 4.22), 340 mμ (log ε 4.22), and 370 mμ (log ε 4.14), and a shoulder at 228 mμ (log ε 4.49); *in 0.1 N ethanolic NaOH:* 225 mμ (log ε 4.51), 256 mμ (log ε 4.43), 296 mμ (log ε 4.50), 385 mμ (log ε 4.34), and shoulders at 230 mμ (log ε 4.52), 320 mμ (log ε 4.31), and 345 mμ (log ε 4.13). *Monoacetate:* m.p. 205–207°C. *Triacetate:* m.p. 248°C. Partial formula (one oxygen unplaced):



Biological activity: Active mainly on gram-positive bacteria. Very slight activity on gram-negative bacteria and *C. albicans* (125 μg per ml), but active on *Ps. pyocyaneus* at 5 μg per ml. Not active on *M. tuberculosis*.

Toxicity: Mice tolerate 1 gm per kg orally.

Reference: 1. Vora, V. C. *et al.* J. Sci. Ind. Research (India) **16C:** 182–185, 1957.

Antibiotic X 464

Produced by: Streptomyces sp.

Method of extraction: Most of the antibiotic present in cells. Extraction from mycelium with methanol, concentration *in vacuo*. Extraction of concentrate with butyl acetate, concentration *in vacuo*. Extraction of residue with petroleum ether, concentration *in vacuo*. Residual oil partitioned between aqueous methanol and petroleum ether. By successive aqueous methanol extraction, most of the activity is collected in the methanol, which is concentrated *in vacuo*. Residue dissolved in benzene and chromatographed over alumina.

Chemical and physical properties: Colorless organic acid; m.p. 170–172°C (decomposition). No characteristic absorption in ultraviolet light. $C_{25}H_{40}O_7$.

Biological activity: Active *in vitro* against gram-positive bacteria and mycobacteria. Not active *in vivo* against bacterial or protozoan infections.

Toxicity: LD_{50} (mice) 2.5 mg per kg intraperitoneally.

Reference: 1. Berger, J. *et al.* J. Am. Chem. Soc. **73:** 5295–5298, 1951.

Antibiotic X 537A

Produced by: Streptomyces sp.

Method of extraction: Most of the antibiotic present in the cells. Extraction of cell material with butyl alcohol, concentration *in vacuo*, washing with sodium carbonate, drying to solid. Extraction of this solid in a Soxhlet apparatus with petroleum ether. Concentration *in vacuo*; crystallization from petroleum ether.

Chemical and physical properties: Colorless organic acid; m.p. 100–109°C. $[\alpha]_D^{26} = -7.2°$ in methanol. Maximal light absorption at 317 and 249 mμ in isopropyl alcohol. Soluble in organic solvents; insoluble in water. Maximal light absorption of the sodium salt at 308 and 245 mμ. $C_{34}H_{52}O_8$.

Biological activity: Active *in vitro* against gram-positive bacteria and mycobacteria. No activity *in vivo* against bacterial or protozoan infections.

Toxicity: LD_{50} (mice) 40 mg per kg intraperitoneally.

Reference: 1. Berger, J. *et al.* J. Am. Chem. Soc. **73:** 5295–5298, 1951.

Antibiotic of Chandrasekhar

Produced by: Streptomyces sp.

Method of extraction: Extraction from broth with ethylene dichloride. Extract dried with Na_2SO_4 and concentrated to dryness under reduced pressure at 40°C.

Chemical and physical properties: Red substance. Sparingly soluble in water; highly soluble in ethanol, butyl alcohol, and ethylene dichloride. Inactivated at 60°C and above.

Biological activity: Active on gram-negative and gram-positive bacteria, including *Ps. pyocyanea* (2 μg per ml) and mycobacteria (5 to 20 μg per ml). Not active on fungi or actinomycetes.

Reference: 1. Chandrasekhar, S. Antibiotics & Chemotherapy **5:** 742–743, 1955.

Antibiotic of Mukherjee

Produced by: Streptomyces sp. resembling the *S. fradiae-S. californicus* group, but different from the neomycin-producer.

Method of extraction: Adsorption on charcoal, elution with 80 per cent acetone (pH 2.2). Acetone evaporated off *in vacuo*. Addition of acetone to the residue precipitates the antibiotic. Recrystallization from water.

Chemical and physical properties: Soluble in water; insoluble in organic solvents. Stable at 100°C for 30 minutes. Not affected by cysteine or acid pH. Chromatography (n-butanol-piperidine *p*-toluenesulfonic acid and methanol-water, 9:1)

indicates that the antibiotic differs from streptomycin, streptothricin, and neomycin. Forms a helianthate.

Biological activity: Culture active on gram-positive and gram-negative bacteria (including streptomycin-resistant *E. coli*), mycobacteria, and fungi. Broth active against gram-positive and gram-negative bacteria.

Reference: 1. Mukherjee, S. K. *et al.* Indian J. Pharm. **15:** 281–282, 1953.

Antibiotic of Rolland

Produced by: Streptomyces sp.

Method of extraction: Extracted from broth with ethyl acetate. Purification by chromatography on alumina.

Chemical and physical properties: Acidic substance. Yellow powder. Soluble in methanol, ethanol, and acetone. Insoluble in water. (Na salt most soluble in water at pH 6.5 to 7.0.) Said to differ from other known antibiotics in ultraviolet absorption and Rf values on chromatography.

Biological activity: Active on gram-positive and certain strains of gram-negative bacteria. Active on mycobacteria, but not pathogenic yeasts or fungi. No cross-resistance with clinically common antibiotics. Active *in vivo* in protecting mice against *D. pneumoniae*, *Streptococcus faecalis*, and *Pr. vulgaris* infections.

Toxicity: LD_{50} (mice) 500 mg per kg intravenously, >4 gm per kg orally.

Reference: 1. Rolland, G. *et al.* Rass. med. sper. **2:** 321–322, 1955.

Antibiotic of Sackmann

Produced by: Streptomyces sp. resembling *S. roseochromogenes.*

Method of extraction: Adsorption on activated carbon, and elution with acidic methanol (pH 3.0). Eluate neutralized and methanol removed by distillation *in vacuo*. Residue precipitated from a solution in warm methanol by addition of anhydrous ether. Purified by hydrochloride → picrate → hydrochloride salt conversion. Purified by chromatography on alumina (acidic) with methanol as solvent and developer. The fastest moving zone, giving a bright blue fluorescence, is the antibiotic. Addition of anhydrous ether to the concentrate of the active fractions precipitates the antibiotic.

Chemical and physical properties: Polypeptide with a reducing sugar moiety. White amorphous powder. Becomes yellow at 144°C, brownish at 169°C, and chars at 235°C. Very soluble in water and acidic (HCl to pH 3) methanol. Not soluble in ether, acetone, isopropyl alcohol, or other organic solvents. Gives an orange color which is quantitative with Weber's reagent. Positive Molisch, Fehling, and biuret reactions. Negative Sakaguchi, maltol, $FeCl_3$, ninhydrin, Millon, Elson-Morgan, Hopkins-Cole, and xanthoproteic tests.

Biological activity: Active on gram-positive bacteria; less active on gram-negative bacteria. Much less active on streptococci than on staphylococci.

Toxicity: LD_{50} (mice) 150 to 160 mg per kg. Nephrotoxic.

Reference: 1. Sackmann, F. Zentr. Bakteriol. Parasitenk., Abt. II **109:** 42–72, 1956.

Antifungal Antibiotic 757

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate extracted with butanol at pH 9.0. Extract concentrated *in vacuo* just until a fine precipitate is formed. Precipitate washed with petroleum ether and re-extracted into aqueous butanol (1:1). Butanol fraction concentrated and new precipitate washed with ether-acetone (4:1).

Chemical and physical properties: Heptaene. Amorphous yellow powder. Soluble in pyridine and NaOH solutions. Slightly soluble in methanol, ethanol, and propylene glycol. Scarcely soluble in butanol. Insoluble in acetone, chloroform, benzol, petroleum ether, and water. Photo-labile. Precipitated by Group 2 metals from aqueous solution. Relatively thermostable. Ultraviolet absorption spectrum maxima (methanol) at 361, 381, and 404 mμ.

Biological activity: Active on yeasts and fungi. Reduces number of seminal cells in germinal tissue of mice and rabbits, and has antimitotic effects on the glandular crypts of mouse intestine and root meristem cells of *Allium cepa*. No activity on Ehrlich adenocarcinoma in mice.

Toxicity: LD_{50} (mice) 5 mg per kg intraperitoneally, 60 mg per kg subcutaneously.

Reference: 1. Craveri, R. and Giolitti, G. Ann. Microbiol. **7:** 81–92, 1956.

Antifungal Antibiotic 7071 R. P.

Produced by: Streptomyces sp. resembling *S. kitasatoensis.*

Method of extraction: Broth-filtrate extracted with butanol. Concentration of the solvent precipitates the antibiotic. Recrystallization from water-saturated butanol.

Chemical and physical properties: Tetraene; m.p. 275–280°C (decomposition). $[\alpha]_D^{20} = +90°$ (c = 1 per cent in methanol); $= +80°$ (c = 1 per cent in

pyridine). C = 58.3%; H = 8.0%; O = 31.5%; N = 1.65%. Ultraviolet absorption spectrum maxima at 291 mμ ($E^{1\%}_{1cm}$ 562), 304 mμ ($E^{1\%}_{1cm}$ 863), and 318 mμ ($E^{1\%}_{1cm}$ 783).

Biological activity: Active on yeasts and fungi. Not active on bacteria. More active than nystatin.

Toxicity: LD_{50} (mice) 37 mg per kg subcutaneously, 250 mg per kg orally.

Reference: 1. Despois, R. *et al.* Giorn. microbiol. **2:** 76–90, 1956.

Antifungal Antibiotic A 228

Produced by: Streptomyces sp. (2).

Method of extraction: I. Filtered broth extracted with n-butanol (or chloroform) at pH 7.0. Extract concentrated under reduced pressure; diethyl or petroleum ether, ethyl acetate, or acetone added to precipitate A 228 complex. Precipitate slurried in ether, then dried *in vacuo*. II. Adsorbed from the whole culture-broth on 1 per cent magnesium trisilicate and 2 per cent siliceous earth, and eluted with acetone, then 80 per cent aqueous acetone. Eluates concentrated under reduced pressure at <35°C to remove the acetone. Aqueous residue extracted with n-butanol. Extract concentrated under reduced pressure. Precipitated from the residue on addition of ether or petroleum ether. Purified by countercurrent distribution (water: n-butanol-diethyl ether, 1:1.75). Two fractions, A 228a and A 228b, are separated by reversed phase partition chromatography on Alloprene (chlorinated rubber containing 2 per cent butanol) with n-butanol-saturated water as solvent and developer. Fraction A 228a is obtained in the earlier fractions, A 228b in the later (2).

Chemical and physical properties: Complex, containing two neutral heptaenes. Yellow-brown substances. Both have the following characteristics: Soluble in methanol, ethanol, and butanol. Soluble in chloroform with loss of biological activity. Very slightly soluble in water. Insoluble in anhydrous acetone, diethyl ether, ethyl acetate, and petroleum ether. Ultraviolet absorption spectrum maxima (aqueous ethanol) at 291, 304, 318, 332, and 350 mμ; $E^{1\%}_{1cm}$: (a) 150, 265, 475, 695, 700, and (b) 160, 290, 520, 785, 805, respectively. Infrared data given in reference 1. Yellowish blue fluorescence in ultraviolet light (aqueous solution). Decolorizes bromine water with the formation of a faintly yellow or white precipitate. Dark violet color in H_2SO_4 . Stable in powder form. In aqueous solution, stable in the cold and comparatively stable at room temperature in the dark. Photo-labile. Alcoholic solutions are more stable than aqueous; more stable at neutrality than at acid pH. C = about 60%; H = about 8%; N = about 2%; S = 4%. No halogens (1, 2).

Biological activity: Active on yeasts and filamentous fungi. Not active on bacteria, mycobacteria, or actinomycetes. Active on *Trichomonas vaginalis* at 15 to 30 μg per ml and *Endamoeba histolytica* at 60 to 120 μg per ml. No difference in antibiotic activity between A 228a and A 228b (2).

Toxicity: Two of three mice were killed by an intraperitoneal injection of 83 mg per kg of A 228a, but 41.5 mg per kg was tolerated. Mice also tolerate 100 mg per kg of A 228b (same route) (2). Not absorbed from the intestinal tract (1).

References:

1. Peynaud, E. and Lafourcade, S. Rev. fermentations et ind. aliment. **8:** 228–242, 1953.
2. Ball, S. *et al.* German Patent 942,047, April 26, 1956.

Antifungal Antibiotic J 4A

Produced by: Streptomyces sp. belonging to *S. fungicidicus* "group G."

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 4.0. Extract concentrated *in vacuo*, and passed through a column of alumina. Water added; solvent removed *in vacuo*. Precipitate filtered off, filtrate adjusted to pH 4.0, and extracted with ethyl acetate. Concentration of extract to yellow syrup. Chromatography on alumina with methanol as solvent and developer. Active fractions concentrated. Antibiotic J 4A precipitated from concentrate in the cold. Recrystallization from ether-methanol. Antibiotic also present in mycelium.

Chemical and physical properties: White prisms; m.p. 164–170°C. Soluble in ethyl acetate and dioxane. Fairly soluble in ethanol, ether, and water. Sparingly soluble in chloroform and petroleum ether. Ultraviolet absorption spectrum maximum at 212 mμ with a minor peak at 260 mμ. C = 61.47%; H = 7.41%; N = 5.15%; O = 25.93%. Negative ninhydrin, Sakaguchi, biuret, Millon, Molisch, Fehling, Seliwanoff, and $FeCl_3$ tests. Weakly positive Tollen test. Gives orange color in 40 per cent H_2SO_4.

Biological activity: Active on fungi, including *Aspergillus* and *Penicillium*, but less active on *Trichophyton*. Not active on the yeasts tested.

Reference: 1. Taguchi, H. and Nakano, A. J. Fermentation Technol. **35:** 145–149, 1957.

Antifungal Antibiotic J 4B

Produced by: Streptomyces sp. belonging to the *S. fungicidicus* "group G."

Method of extraction: Broth-filtrate extracted first with ethyl acetate at pH 4.0 to remove antifungal antibiotic J 4A and antibiotic J 4, then with butanol at pH 8.0. Extract concentrated *in vacuo*. Residual solution taken up in methanol. Addition of ether precipitates J 4B. Antibiotic also present in mycelium.

Chemical and physical properties: Tetraene. Yellow powder. Insoluble in ether, petroleum ether, and chloroform. Positive Sakaguchi reaction. Negative ninhydrin, biuret, Millon, Molisch, Seliwanoff, and $FeCl_3$ tests; weakly positive Tollen test. Gives a brown color in 40 per cent H_2SO_4. Ultraviolet absorption spectrum maxima at about 288, 303, and 311 mμ.

Biological activity: Active on yeasts and filamentous fungi. Not active on bacteria.

Reference: 1. Taguchi, H. and Nakano, A. J. Fermentation Technol. **35:** 145–149, 1957.

Antifungal Antibiotics of Rao and Uma

Produced by: Streptomyces spp. related to *S. viridans* (1).

Method of extraction: Extracted with n-butanol from broths and mycelium (1).

Chemical and physical characteristics: Three antibiotics, A, B, and C, have Rf values of 0.00, 0.33, and 0.95, respectively, on paper chromatography (benzene-acetic acid-water, 2:2:1). *A* and *B* have ultraviolet absorption spectrum maxima at 360, 380, and 405 mμ, indicating that they are heptaenes. *C* has no characteristic absorption from 250 to 400 mμ (1).

Biological activity: Active on yeasts and probably on other fungi. Not active on bacteria (1).

Reference: 1. Rao, P. L. N. and Uma, B. N. Nature, London **182:** 115–116, 1958.

Antifungal Heptaene F 17C

Produced by: Streptomyces cinnamomeus f. *azacoluta*.

Method of extraction: Extraction of mycelium with 95 per cent ethanol. Extract concentrated *in vacuo* under nitrogen. Precipitate collected and washed with water. Precipitate dissolved in n-butanol-pyridine-water (1:1:2) and added to n-butanol-water (7:8). Emulsion is centrifuged. Concentration of the upper phase under nitrogen and *in vacuo* to three-fifths of the original volume. The polyene precipitates and is washed and dried.

Chemical and physical properties: Amphoteric heptaene. Amorphous yellow powder. Deep blue color with sulfuric acid. Absorption of light similar to candidin, with a maximum at 335 mμ in aqueous neutral solutions. In ethanol, peaks at 408, 383, 365, and 347 mμ (weak) with a shoulder at 320 mμ. Insoluble in ether, petroleum ether, benzene, chloroform, ethyl acetate, and water. Soluble in pyridine, methanol, and dimethyl sulfoxide. Slightly soluble in absolute ethanol and acetone; solubility increases upon addition of water to these solvents. Unstable: half-life of neutral aqueous solutions 1 hour at 70°C, of neutral 95 per cent ethanolic solutions 4 to 6 days at 4°C in the dark. Paper chromatography suggests that F 17C has one factor in common with ascosin and another one with PA 150 and the antifungal antibiotic 757.

Biological activity: Active against yeasts and filamentous fungi. Inactive against bacteria and actinomycetes.

Reference: 1. Craveri, R. *et al.* Antibiotics & Chemotherapy **10:** 430–439, 1960.

Antimycins

Produced by: Streptomyces sp. (1, 15, 18, 25, 27), *S. kitasawaensis* (17), and *S. griseus* (27).

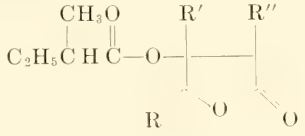
Synonyms: Antimycin A, antimycin A 35 (1, 15), antimycin A 102 (15), antipiriculin A (17), virosin (19), antibiotic 720A (18). See also blastmycin.

Remarks: Like the actinomycins, the antimycin complexes contain a number of closely related substances. The complex produced by one organism may contain the same components as the complex produced by another, but in different proportions. One complex may contain components not found in another.

Method of extraction: Broth, adjusted to pH 9.0, treated with Celite 503, filtered, and filtrate adjusted to pH 2.5 and retreated with Celite. Celite washed with acidic water and eluted with 95 per cent ethanol. Ethanol concentrated *in vacuo*, and extracted successively with chloroform. Chloroform extract concentrated *in vacuo* to a dark oil. Oil extracted with benzene. Benzene equilibrated with an equal volume of 70 per cent ethanol. Benzene layer, after separation, concentrated *in vacuo* to a dark oil. Oil stirred with petroleum ether, and the brown solid that separates is extracted exhaustively with petroleum ether in a Soxhlet apparatus. Resulting light tan solid crystallized from ether, and recrystallized from methanol and benzene-petroleum ether. Further purification by countercurrent distribution and partition chromatography (10, 23, 24).

Chemical and physical properties: All the antimycin components have the following general properties: Colorless substances. Soluble in methanol, ethanol, acetone, ether, n-butanol, chloroform, and ethyl acetate. Slightly soluble in petroleum ether, benzene, carbon disulfide, carbon

tetrachloride. Insoluble in water, 5 per cent HCl, and 5 per cent $NaHCO_3$ (3, 12, 15, 17). Ultraviolet absorption spectrum maxima at about 225 to 228 and 320 to 330. Bathochromic shift on addition of base. Infrared spectrum (the same for all components isolated) given in references 12 and 24. Positive hydroxamic, Millon, $FeCl_3$, Gibbs diazo, Liebermann's nitroso, and $KMnO_4$ tests. No color in concentrated H_2SO_4. Negative Molisch, ninhydrin, Ehrlich, fuchsin aldehyde, 2,4-dinitrophenylhydrazine, chromotropic acid, pine splint, and cyanogen bromide tests (3, 12, 17). Stable at room temperature. Photosensitive (23). Mild alkaline hydrolysis yields, among other products, antimycic acid ($C_{11}H_{14}O_5N_2$) and N-(3-aminosalicyloyl)-L-threonine (12, 13). Hydrolysis products also include a neutral, stable, colorless, pleasant-smelling, water-insoluble oil, $C_{16}H_{28}O_4$ (26), believed to be:



Individual components of the antimycin complexes all contain the antimycic acid residue, but differ in the nature of the substitution in the neutral fragment above (*i.e.*, R, R′, and R″ = H or alkyl group) (28). Two different structures have been proposed for "antimycin," but for *which* antimycin component is not clear (20, 26). One complex (A 35) has been separated into five fractions: A_1, A_{2a}, A_{2b}, A_3, and a minor fraction, A_4 (23). Another (A 102) contained almost 60 per cent A_3, in addition to A_1, A_2, and A_4 (15, 23). Another complex was shown to contain the same components as A 102 (24). Rf values of these various components on paper chromatography in different systems are given in references 23 and 24. *Antimycin* A_1: m.p. 149–150°C (23) or 147–148°C (24). $[\alpha]_D = +74.0°$ (in chloroform). C = 61.11%; H = 7.32%; N = 5.03%. $C_{28}H_{40}N_2O_9$ (23, 24). *Antimycin* A_{2a}: m.p. 147–148°C. $C_{25}H_{34}O_9N_2$. *Antimycin* A_{2b}: m.p. 168°C. $C_{25}H_{30}O_9N_2$ (23). *Antimycin* A_3. m.p. 170.5–171.5°C (23) or 167–168°C. $[\alpha]_D = +84.0°$ (in chloroform). C = 60.03%; H = 6.93%; N = 5.33%. $C_{26}H_{36}N_2O_9$ (23, 24).

Biological activity: Active against yeasts and filamentous fungi. Very little activity against bacteria (1). Inhibits influenza virus in tissue culture by action of the host tissue cells (8) or by direct inactivation of the virus (21). Slightly active on RC mammary carcinoma in mice (22). Selectively inhibits an electron transport component acting between succinic dehydrogenase and cytochrome C in the succinoxidase system, and between diaphorase and cytochrome C in diphosphopyridine nucleotide systems (4, 5, 9, 11). Partially inhibits O_2 uptake in diphosphopyridine nucleotide-coupled oxidation of malate and D-glyceraldehyde 3-phosphate in certain organs (14). Some protective action on apple scab and tomato early blight (2), *Helminthosporium* seedling blight of oats (15), and rice blast (*Piricularia oryzae*) (17, 19).

Toxicity: Complexes A 35 and A 102: LD_{50} (mice) 0.9 mg per kg intravenously, 7.6 mg per kg intraperitoneally, and 25 mg per kg subcutaneously (15, 26). LD_{50} (rats) 0.81 mg per kg intraperitoneally. LD_0 (rats) 12 mg per kg, and LD_{100} (rats) 30 mg per kg orally (11, 15). Toxic to certain insects and spiders (6, 7). Not toxic to a variety of plants when applied as a spray (100 units per ml) (2). Greatest phytotoxicity in most rapidly growing parts of plant, and most toxic in oil solution (15).

Utilization: Useful in elucidation of metabolic processes.

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Antimycoin

Produced by: Streptomyces aureus (1).

Synonyms: Fungicidin RAW; antibiotic C 381.

Remarks: Following the original description (1) of this antibiotic, the culture ceased to produce the polyene. It was later found that production of one of the components of the original complex could be induced by the addition of high concentrations of $CaCl_2$ or $MgCl_2$ and mevalonic acid to the culture medium (4, 5).

Method of extraction: Broth extracted with butanol. Extract cooled to precipitate lipid impurities, then concentrated to dryness *in vacuo*. Residue washed with petroleum ether, then acetone, and dried. Residue can also be taken up in ethanol, filtered, and distilled to dryness. Precipitates from an ethanol solution on addition of ice-cold ether (1).

Chemical and physical properties: Tetraene. Original complex (see "Remarks") contains two active components, Rf values about 0.55 and 0.45 (ethanol- n-butanol-water, 1:5:5). The faster moving component is called antimycoin A (4, 5). *Complex:* Soluble in water, ethanol, and physiological saline solution. Insoluble in ether, chloroform, and acetone. Unstable at acid pH; most stable at pH 7.0. Ultraviolet absorption spectrum maxima at 290, 305, and 316 mμ (ethanol) (1, 4). Differs from nystatin and rimocidin (1, 3). *Crude antimycoin A:* Brown or gray substance. Soluble in pyridine, butanol, and dimethyl sulfoxide (5).

Biological activity: Active on yeasts and fungi, including *Cryptococcus*. Not active on bacteria or actinomycetes. Prevents *C. albicans* infection and has some activity on *Histoplasma capsulatum in ovo*. Active *in vivo* (mice) on *Coccidioides immitis* infections (1, 2).

Toxicity: LD_{50} (mice) 204 mg per kg intraperitoneally (1), >532 mg per kg subcutaneously (2).

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3. Oroshnik, W. *et al.* Science **121:** 147–149, 1955.
4. Schaffner, C. P. *et al.* Antibiotics Ann. 869–873, 1957–1958.
5. Steinman, I. D. Thesis, Rutgers University, 1958.

Antiphlei Antibiotic I

Produced by: Streptomyces sp. resembling *S. aureus*.

Method of extraction: Broth adjusted to pH 5.0 and filtered. Filtrate evaporated *in vacuo* to dryness and extracted with methanol. Extract evaporated to dryness and re-extracted into methanol. Addition of butanol in excess, concentration *in vacuo* of supernatant, and precipitation with acetone. Reprecipitated from methanol with acetone. Purified by conversion to helianthate, then hydrochloride.

Chemical and physical properties: Basic substance. *HCl salt:* White amorphous powder. *Helianthate:* Crystalline; m.p. 243–247°C. (decomposition). Negative biuret, xanthoproteic, Millon, Sakaguchi, Vole sulfur, Hopkins-Cole, Molisch, glucosamine, and maltol tests. Most stable to boiling at pH 5 to 6; less stable at alkaline than at acid pH. Soluble in anhydrous methanol.

Biological activity: Active mainly on *M. phlei*. Less active on *M. tuberculosis* (human type), *M. smegmatis*, and *M. avium*. Very slight activity on *B. subtilis* and *B. anthracis*. No activity on gram-negative bacteria.

Toxicity: Mice tolerate 10 to 20 mg of the crude HCl salt intramuscularly and intravenously.

Reference: 1. Ouchi, N. Tôhoku J. Exptl. Med. **55:** 355–365, 1952.

Antiphlei Antibiotic II

Produced by: Streptomyces aureus (2).

Synonym: New antiphlei factor (2).

Method of extraction: Broth-filtrate evaporated *in vacuo* at pH 5.0, dried, and extracted with anhydrous methanol. Addition of butanol to extract precipitates impurities; subsequent addition of acetone to the methanol precipitates the antibiotic (1).

Chemical and physical properties: Crystalline helianthate; m.p. 243–247°C (1).

Biological activity: Active on mycobacteria (1). Not active on other bacteria (2).

Toxicity: Doses of 10 to 20 mg in mice (intravenously and intramuscularly) are nontoxic (1).

References:

1. Ouchi, N. Tohoku J. Exptl. Med. **54:** 144, 1951.
2. Kurosawa, H. J. Antibiotics (Japan) **4:** 183–193, 1951.

Antismegmatis Antibiotic

Produced by: Streptomyces sp. resembling *S. lavendulae.*

Method of extraction: Concentration by precipitation by cold.

Chemical and physical properties: Heat-stable at pH 7.0.

Biological activity: Most active, at an alkaline reaction, against *M. smegmatis* and *M. phlei.* No activity against bacteria, fungi, or a pathogenic strain of *M. bovis.*

Reference: 1. Kelner, A. and Morton, H. E. Proc. Soc. Exptl. Biol. Med. **63:** 227–230, 1946.

Antitumor Antibiotic 289

Produced by: Streptomyces sp. (1) resembling *S. tanashiensis* (luteomycin-producer) (3).

Synonym: Said to resemble luteomycin (1), but differs in ultraviolet absorption spectrum, elementary analysis, and biological activity (3).

Method of extraction: I. Broth-filtrate extracted with ethyl or butyl acetate at pH 7.0. Re-extracted into water at pH 2.0. Process repeated twice using less solvent each time. Adjusted to pH 5.0 and freeze dried. II. Adsorption on diatomaceous earth from water or butyl acetate. Eluted with acetone (1).

Chemical and physical properties: Basic substance. Orange or reddish yellow powder. *HCl salt:* Soluble in water, methanol, ethanol, and acetone. *Sulfate:* Orange or orange-brown crystals. Soluble in water. Slightly soluble in ethanol and acetone. Forms a picrate, reineckate, helianthate, and citrate. Positive $FeCl_3$ test. Negative ninhydrin, Molisch, and Sakaguchi tests. Indicator properties: Changes from orange-yellow to purple from pH 7.5 to 9.0. Ultraviolet absorption spectrum (at acid pH) maxima at 215, 257, and 430 mμ (1). At alkaline pH, the peaks shift toward the longer wave lengths. *Sulfate:* C = 48.21%; H = 5.40%; N = 2.21%; S = 4.97%. $C_{26}H_{33}NO_{12} \cdot H_2SO_4$. Most stable at pH 3 to 6. Less stable at alkaline pH. Thermolabile (2). Acid hydrolysis product is biologically active "teomycic acid," $C_{17}H_{23}HO_7$, which is a green-black substance with ultraviolet absorption spectrum maxima at 258 to 261 mμ ($E^{1\%}_{1cm}$ 485) and 355 to 356 mμ ($E^{1\%}_{1cm}$ 151) (methanol) and no melting point up to 300°C. Other data given in reference 5.

Biological activity: Active on gram-positive bacteria, but less so than luteomycin. Very little or no activity on fungi and gram-negative bacteria. Active on *Toxoplasma gondii in vitro* (4). Active *in vivo* (rats) against Yoshida sarcoma (1). Antimitotic effect (2). Kills HeLa cells at 10 μg per ml and causes disappearance of HeLa cells in mitosis at 1.25 μg per ml (6). Teomycic acid (hydrolysis product) moderately active on gram-positive bacteria, including mycobacteria (3 to 50 μg per ml). No activity on fungi or gram-negative bacteria. No activity on Ehrlich ascites carcinoma (5).

Toxicity: MLD (mice) about 10 mg per kg intravenously (1).

References:

1. Umezawa, H. *et al.* J. Antibiotics (Japan) **6A:** 45–51, 1953.
2. Osato, T. *et al.* J. Antibiotics (Japan) **6A:** 52–56, 1953.
3. Okami, Y. *et al.* J. Antibiotics (Japan) **6A:** 153–157, 1953.
4. Okami, Y. *et al.* J. Antibiotics (Japan) **8A:** 126–131, 1955.
5. Nakamura, S. J. Antibiotics (Japan) **9A:** 207–209, 1956.
6. Umezawa, H. Giorn. microbiol. **2:** 160–193, 1956.

Antitumor Substance 1418 A1

Produced by: Streptomyces sp.

Remarks: Not sufficiently characterized to permit differentiation from certain other antibiotics having antitumor activity and ultraviolet spectra of a similar nature. Authors (1) state that it differs from cellocidin and lenamycin.

Method of extraction: Extraction of broth-filtrate at pH 2.0 or 7.0 with butanol. Extract evaporated, residual syrup washed with ether. Taken up in benzene, concentrated, and filtered to remove white precipitate of trans-cinnamic acid amide. Subjected to countercurrent distribution (76 per cent methanol-benzene-chloroform, 2:1:1).

Chemical and physical properties: Powder. Soluble in methanol, ethanol, acetone, ethyl acetate, chloroform, carbon tetrachloride, and benzene. Slightly soluble in ether and ethyl Cellosolve. Insoluble in water. Ultraviolet absorption spectrum maxima at 217 mμ (methanol) or at 225 and 337.5 mμ (alkaline aqueous solution). Infrared spectrum given in reference 1. Rf values given in reference 1. Stable in the culture broth at pH 2 to 7, but unstable to pH 8.0.

Biological activity: Active on Ehrlich carcinoma and HeLa cells *in vitro*. Inhibited increase of ascites and prolonged survival of mice with Ehrlich ascites carcinoma.

Toxicity: LD_{50} 2.5 to 5.0 mg per kg intravenously. Mice tolerate 2.5 mg per kg intraperitoneally.

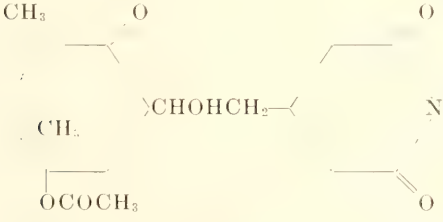
Reference: 1. Murase, M. *et al.* J. Antibiotics (Japan) **12A:** 75–80, 1959.

Antitumor Antibiotic E 73

Produced by: Streptomyces albulus. This culture produces nystatin and two forms of cycloheximide, in addition to E 73.

Remarks: Physical and some chemical properties of E 73 are said to resemble cycloheximide and the streptovitacins.

Chemical and physical properties: Colorless crystals. $C_{17}H_{25}O_6N$. 3-[2-(3,5-Dimethyl-5-acetoxy-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide. Degradation products given in reference 2. Structural formula:



Biological activity: Active on sarcoma 180 in mice (1).

References:

1. Rao, K. V. and Cullen, W. P. Abstr. 134th Meeting Am. Chem Soc. 22-O to 23-O, 1958.
2. Rao, K. V. Abstr. 134th Meeting Am. Chem. Soc. 23-O, 1958.
3. Rao, K. V. *et al.* J. Am. Chem. Soc. **82:** 1127–1132, 1960.

Antivirubin

Produced by: Streptomyces longispororuber (2).

Chemical and physical properties: Pigment (1).

Biological activity: Active against gram-positive bacteria (2) and against influenza, vaccinia, and tobacco mosaic viruses *in vitro* (1).

Toxicity: Said to be toxic (1).

References:

1. Germanova, K. I. Voprosy Virusol. 4(1): 71–76, 1959.
2. Tremina, G. A. Antibiotiki 1(4): 9–13, 1956.

Ascosin

Produced by: Streptomyces canescus (1, 5).

Synonyms: Similar to trichomycin and candicidin.

Method of extraction: Mycelium extracted with methanol, pyridine, or quinoline (1). Broth extracted with butanol at pH 7 to 8. Heptane or "Stoddard solvent" added to extract at pH 7 to 8, followed by solid $NaHCO_3$ (8 gm per gallon); pH adjusted to 9.5 to 10.5 with NaOH. Aqueous layer cooled, then adjusted to pH 4.0 to precipitate ascosin (5).

Chemical and physical properties: Contains two heptane components, A (ethanol-soluble) and B (ethanol-insoluble). *Component A:* Ultraviolet absorption spectrum maxima at 340, 358, 377, and 399 mμ. *Component B:* maxima at 340, 358, 376, and 398 mμ (in ethanol). In water, the last three peaks are flattened to form a plateau at 320 to 350 mμ (4). *Crude substance:* Orange-brown or yellow-orange. Weakly acid, unstable. Soluble in water-containing solvents such as pyridine, picolines, and quinoline. Slightly soluble in water, dry pyridine, dry quinoline, phenol, methanol, ethanol, butanol, formamide, ethyl acetate, n-butyl acetate, and amyl acetate. Scarcely soluble or insoluble in petroleum ether, benzene, chloroform, acetone, ether, dioxane, and acetic anhydride. Soluble in, but inactivated by H_3PO_4, dipropyl hydrogen phosphate, and aromatic sulfonic acids (1, 5). Gives precipitates with Ag^+, Ba^{++}, Fe^{+++}, and aqueous, but not methanolic brucine. Intense unstable blue color in 35 per cent H_3PO_4 reversed by dilution with water or methanol. Gives a green color with HCl (5). Negative Molisch, Tollen, Ehrlich, $FeCl_3$, ninhydrin, Benedict, and Sakaguchi tests. Crude material has ultraviolet absorption spectrum maxima at 234, 240 (infl.), 288, and 326 mμ (infl.), in addition to those given above (1). Infrared data given in reference 1 and spectrum in reference 5.

Biological activity: Active on yeasts and certain filamentous fungi (*e.g.*, *P. spinulosum* but not *P. patulum* or *P. chrysogenum*; and *A. niger* but not other aspergilli tested) (1). Active on the yeast phase of *Histoplasma capsulatum*, but not

the mycelial phase (5). Active *in vivo* (mice) on experimental histoplasmosis but not torulosis (*Cryptococcus neoformans*) (1, 3). Antifungal activity *in vitro* suppressed by unsaturated, but not saturated, fatty acids, and Tween 80 (2).

Toxicity: LD_{50} (mice) 8.6 mg per kg (crude substance) (1), or 18 to 22 mg per kg intraperitoneally (5), 12.5 mg per kg intravenously. Intravenous administration accompanied by vestibular disturbances and nerve cell degeneration in brain nuclei (1).

Utilization: Active on tinea capitis in children (6).

References:

1. Hickey, R. J. *et al.* Antibiotics & Chemotherapy **2:** 472–483, 1952.
2. Hickey, R. J. Arch. Biochem. Biophys. **46:** 331–336, 1953.
3. Emmons, C. W. and Haberman, R. T. Antibiotics & Chemotherapy **3:** 1204–1210, 1953.
4. Vining, L. C. *et al.* 8th Congr. intern. botan., Paris Vol. prelim. Sect. 24, 106–110, 1954.
5. Cohen, I. R. U. S. Patent 2,723,216, November 8, 1955.
6. Lubowe, I. I. *et al.* Antibiotics Ann. 135–139, 1956–1957.

Aspartocin

Produced by: Streptomyces griseus var. *spiralis*, *S. violaceus*.

Synonym: Similar to amphomycin.

Method of extraction: To the culture are added 1 gm of calcium chloride per liter and some Hyflo Super-Cel. pH adjusted to 5.0 to 5.5 with hydrochloric acid; the mixture then stirred and filtered. Cake is washed with water and suspended in water, the pH of which is adjusted to 9.7 to 10. Insoluble materials discarded. Alkaline extract adjusted to pH 1.0 to 2.0 and extracted with n-butanol. Neutralized butanol is concentrated, and calcium chloride added. pH adjusted to 5.0 to 5.5, and the white microcrystalline calcium salt of aspartocin removed by centrifugation, washed with wet butanol, then washed with acetone, and air dried (1).

Chemical and physical properties: Acidic polypeptide with a fatty acid moiety. C = 53.36%; H = 7.51%; N = 13.36%; S = 0.42%; Cl = 0.07%; ash = 0.62%; NH_2 (Van Slyke) = 4.27%. No characteristic light-absorption spectra. $[\alpha]_D^{25}$ = +26.4° (c = 2.1 per cent in methanol). More than 50 mg per ml of aspartocin will go into solution in methanol, ethanol, or glacial acetic acid. Dissolves slowly in water or n-butanol. Relatively insoluble in acetone or ethyl acetate. More readily soluble in water at pH <3.0 or >3.6. At pH 3.3, calcium chloride helps to dissolve the antibiotic, and sodium chloride precipitates it. At pH 3, 6 per cent of the activity is left after 30 minutes at 100°C; at pH 5.0, 72 per cent of the activity remains. Other pH values are intermediate. Aspartocin can be separated from amphomycin by chromatography and electrophoresis. Positive biuret test. Rapid uptake of bromine and decolorization of potassium permanganate. Negative Millon, xanthoproteic, Sakaguchi, tryptophan (Tillman-Alt), sodium nitroprusside, Molisch, and anthrone tests. Acid hydrolysates ninhydrin-positive and contain an oil which has the properties of an unsaturated fatty acid. Paper chromatography revealed seven ninhydrin-positive components in the acid hydrolysate. Four were identified by bioassays and paper chromatography as aspartic acid (35 per cent), glycine (10 per cent), L-proline (8 per cent), and L-valine (8 per cent). Also characterized by the hydrolysates were: D-α-pipecolic acid, α(L)β-methyl aspartic acid, and α-β-diaminobutyric acid (4).

Biological activity: Active against gram-positive but not against gram-negative bacteria. Four to eight times more active than amphomycin against *B. subtilis* and *Corynebacterium xerosis*. Activity reduced *in vitro* by inorganic phosphates, and increased by calcium ions. Development of resistance slow. Cross-resistance with amphomycin (2). Effective in mice infected with *Staph. aureus*, *Streptococcus pyogenes*, and *D. pneumoniae* when administered intraperitoneally, subcutaneously, or intravenously; not effective orally (3).

Toxicity: LD_{50} (mice) 110 mg per kg intraperitoneally, 200 mg per kg subcutaneously, 120 mg per kg intravenously (3).

References:

1. Shay, A. J. *et al.* Antibiotics Ann. 194–198, 1959–1960.
2. Kirsch, E. J. *et al.* Antibiotics Ann. 205–212, 1959–1960.
3. Redin, G. S. and McCoy, M. E. Antibiotics Ann. 213–219, 1959–1960.
4. Martin, J. H. *et al.* J. Am. Chem. Soc. **82:** 2079, 1960.

Aureofacin

Produced by: Streptomyces aureofaciens. This culture also produces chlortetracycline.

Synonyms: Probably the same as antibiotic AYF and ayfactin.

Method of extraction: Mycelium extracted with methanol. Extracts combined and concentrated *in vacuo* to precipitate aureofacin.

Chemical and physical properties: Heptaene.

Yellowish brown powder. Soluble in alcohols, acetone, glacial acetic acid, methyl Cellosolve, and ethylene glycol. Slightly soluble in benzene and dioxane; insoluble in water at any pH, chloroform, esters, ether, and petroleum ether. Ultraviolet absorption spectrum maxima at 359, 380, and 402 mμ. Slightly positive Molisch and Fehling tests. Negative $FeCl_3$ test. Blue-violet color in concentrated H_2SO_4. Unstable at acid pH. Does not contain S.

Biological activity: Active on fungi and yeasts. Activity affected by cysteine but not glucose.

Toxicity: LD_{50} (mice) 5 mg per kg intraperitoneally.

Reference: 1. Igarasi, S. *et al.* J. Antibiotics (Japan) **9B:** 79–80, 1956.

Aureolic Acid

Produced by: Streptomyces sp.

Method of extraction: Extraction of broth-filtrate with a mixture of butanol and chloroform. Concentration of solvent *in vacuo*. Concentrate applied to a column of Florisil which is washed with chloroform to remove impurities. Elution of antibiotic with 20 per cent methanol in chloroform. Concentration *in vacuo* to amorphous yellow-tan powder. Powder is dissolved in methanol; upon addition of chloroform, crystallization of a magnesium salt of the acid begins.

Chemical and physical properties: Weak acid, yellow. Tentative formula of magnesium salt: $(C_{56\text{-}60}H_{96\text{-}104}O_{29\text{-}31})_2$ Mg. $[\alpha]_D = +68°$ (1 per cent in methanol). Soluble in lower alcohols and acetone; moderately soluble in water, ethyl acetate, and ether. Negative Fehling and anthrone tests; not decolorized by sodium hydrosulfide. Positive $FeCl_3$ test; positive test with tyrosine reagent. Ultraviolet light-absorption maxima for the magnesium salt in 0.01 *N* NaOH at 235 and 280 mμ.

Biological activity: Active against certain gram-positive bacteria; limited activity against *Trichomonas vaginalis*. No activity against gram-negative bacteria, mycobacteria, fungi, or viruses. Limited activity against *Streptococcus pyogenes in vivo*.

Toxicity: LD_{50} (mice) 2.5 to 5 mg per kg intravenously. A dose of 0.25 mg per kg is fatal to rabbits and dogs. Low oral toxicity, suggesting lack of absorption.

Reference: 1. Grundy, W. E. *et al.* Antibiotics & Chemotherapy **3:** 1215–1220, 1953.

Aureothricin

Produced by: Streptomyces thioluteus (6), *S. farcinicus* (4), *S. celluloflavus* (7), *S. cyanoflavus* (16), and other *Streptomyces* spp. (8, 15).

Synonym: Farcinicin (4).

Remarks: Has the nucleus 3-amino-5-methylpyrrolin-4-ono-(4,3-D)-1,2-dithiole in common with thiolutin (11). Reported to be produced simultaneously with thiolutin by one of these cultures (15).

Method of extraction: Ethyl acetate extract of broth and mycelium concentrated *in vacuo*, dehydrated with anhydrous Na_2SO_4, and chromatographed on an alumina column. Column developed with ethyl acetate-ether (1:1). Active yellow fraction concentrated *in vacuo* until crystals appear, then chilled for 24 hours. Recrystallized from ethyl acetate (1, 11).

Chemical and physical properties: Golden-yellow "thready" crystals (1). Sublimes at 200°C; decomposes at 254°C (uncorrected) (2, 3) or m.p. 260–270°C (decomposition) (9). Insoluble in water; slightly soluble in ethyl acetate, butyl acetate, acetone, benzol, ether, and ethanol. λ_{max} 248 (ϵ = 6100), 312 (ϵ = 3900), and 388 (ϵ = 11,000). Infrared spectrum data given in reference 5. Green color in concentrated HCl after 24 hours. $C_9H_{10}N_2O_2S_2$ (1, 2, 3, 5, 9). Structural formula (9) given in Chapter 6. 3-Propionamido derivative of 3-amino-5-methylpyrrolin-4-ono-(4,3-D)-1,2-dithiole. Acid hydrolysis yields pyrrothine, $C_6H_6N_2OS_2$, a weak amine isolated as the hydrochloride (pK_a' 2.9), which gives a red color with glutaconic aldehyde and is also a hydrolysis product of thiolutin (11).

Biological activity: Active on gram-positive and gram-negative bacteria (2), as well as a variety of fungi and bacteria pathogenic for plants (13). Inhibits growth of ascites cells of Ehrlich carcinoma in mice, but does not prolong the survival period (12). Promotes chick growth at 15 mg per kg of ration (10).

Toxicity: More toxic than chloramphenicol (2). Kills HeLa cells at 2.5 μg per ml (14).

References:

1. Maeda, K. Japan. Med. J. **2:** 85–88, 1949.
2. Umezawa, H. *et al.* J. Antibiotics (Japan) **2:** 107–111, 1949.
3. Maeda, K J. Antibiotics (Japan) **2:** 795–796, 1949.
4. Hata, T. *et al.* J. Antibiotics (Japan) **3:** 312–325, 1950.
5. Celmer, W. D. *et al.* J. Am. Chem. Soc. **74:** 6304–6305, 1952.
6. Okami, Y. Thesis, Hokkaido University, 1952 (as given in Washizu, F. *et al.* J. Antibiotics (Japan) **7A:** 60, 1954).
7. Nishimura, H. *et al.* J. Antibiotics (Japan) **6A:** 57–65, 1953.

8. Maeda, K. J. Antibiotics (Japan) **6A:** 137–138, 1953.
9. Celmer, W. D. and Solomons, I. A. Antibiotics Ann. 622–625, 1953–1954.
10. Takahashi, T. *et al.* J. Antibiotics (Japan) **7A:** 26, 1954.
11. Celmer, W. D. and Solomons, I. A. J. Am. Chem. Soc. **77:** 2861–2865, 1955.
12. Nitta, K. *et al.* J. Antibiotics (Japan) **8A:** 120–125, 1955.
13. Koaze, Y. *et al.* J. Antibiotics (Japan) **9A:** 89–96, 1956.
14. Umezawa, H. Giorn. microbiol. **2:** 160–193, 1956.
15. Nakamura, M. *et al.* Ann. Rept. Takamine Lab. **9:** 35–43, 1957.
16. Funaki, M. *et al.* J. Antibiotics (Japan) **11A:** 143–149, 1958.

Ayfactin

Produced by: Strains of *Streptomyces aureofaciens* and *S. viridifaciens* which produce tetracycline and/or chlortetracycline.

Synonyms: Antibiotic AYF and aureofacin are probably identical with ayfactin.

Method of extraction: I. Whole broth acidified to pH 2.0 and filtered with a filter-aid. Mycelium adjusted to pH 9.0 to 10.0 with NH_4OH and extracted with n-butanol. Extract washed with water, adjusted to pH 7.0, and concentrated by azeotropic distillation, preferably *in vacuo*. Ayfactin precipitates on cooling or on addition of Skellysolve C. Purified by: (a) Slurrying with methyl isobutyl ketone and water. Precipitate taken up in pyridine, solution concentrated to dryness *in vacuo*, and toluene added to precipitate ayfactin from the residue. Precipitate dissolved in dimethylformamide and filtered to remove insoluble impurities. Precipitated from filtrate with Skellysolves, isopropanol, or toluene. (b) Slurrying with aqueous acid, hot formamide, methanol, or acetone. II. Mycelium obtained as in I; extracted with acetone. Extract adjusted to pH 6.0 to precipitate ayfactin. III. Purified by suspending crude substance in a water-n-butanol (1:1) mixture, adjusting to pH 1.5 with stirring, and collecting the active precipitate formed at the interface. Butanol-phase extracted with water at pH 7.0. Aqueous extract lyophilized to give ayfactin. Butanol concentrated, and ayfactin precipitated on addition of Skellysolve C.

Chemical and physical properties: Crystalline heptaene. Does not sublime *in vacuo* up to 200°C. Nearly insoluble in water. Very soluble in n-butanol, dimethylformamide, pyridine, morpholine, and piperidine. Insoluble in ethyl acetate, acetone, ether, methanol, chloroform, and HCl. Ultraviolet absorption spectrum maxima (pyridine) at 330, 348, 366, 388, and 410 mμ. Infrared spectrum given in reference 1. C = 64.85%; H = 7.68%; N = 3.01%. $C_{35}H_{55-56}NO_7$.

Biological activity: Probably active on filamentous fungi and yeasts. Little antibacterial activity. Active in protecting mice from *C. albicans* infections. Not absorbed from the gastrointestinal tract.

Toxicity: LD_{50} (mice) 0.8 mg per kg intraperitoneally.

Reference: 1. British Patent 796,982, June 25, 1958.

Azalomycins

Produced by: Streptomyces hygroscopicus K5-4, which also produces an antimycobacterial factor.

Synonyms: Azalomycin F has properties in common with musarin and hygrostatin. Azaolomycin B is similar to elaiophylin.

Method of extraction: I. Extraction of the wet mycelial cake with acetone. Evaporation of acetone *in vacuo*. Residue dissolved in methanol, which is poured into 5 times its volume of ether. The insoluble part is mainly azalomycin F; the fraction soluble in the methanol-ether mixture is mainly azalomycin B and the antimycobacterial factor. II. Extraction of the broth-filtrate with ethyl acetate yields azalomycin B; extraction with butanol yields azalomycin F (1). *Azalomycin B*, containing solvent solution from either the mycelium or the broth, is concentrated to dryness *in vacuo*, dissolved in ethyl acetate, washed with 2 per cent $NaHCO_3$, 0.01 *N* HCl, and water, and concentrated *in vacuo*. Upon standing overnight at 5–10°C, crystals of azalomycin B precipitate. Recrystallization from aqueous alcohol or acetone (1). *Azalomycin F:* Crude extract of azalomycin F is dissolved in methanol and chromatographed over a column of alumina. Elution with methanol or 20 per cent aqueous methanol. Eluate is concentrated. Upon addition of acetone, the antibiotic precipitates. Recrystallization from methanol-acetone solutions and finally from methanol (1).

Chemical and physical properties: Azalomycin B: Neutral compound. White, needle-shaped crystals; m.p. 185–187°C (decomposition). C = 61.88%; H = 8.72%; OCH_3 = 10.12%. Molecular weight (Rast) 284. $C_{14}H_{24}O_5$. Molecular weight 272.33. $[\alpha]_D^{25} = -48°$ (c = 1 per cent in methanol). Light-absorption maximum 252.5 mμ in methanol. Infrared absorption spectrum given in reference

2. Soluble in methanol, ethanol, and chloroform. Moderately soluble in acetone and ethyl acetate. Slightly soluble in ether and benzene. Insoluble in water and petroleum ether. Dark brownish color in Tollen reaction, with no mirror. Green color in Fehling test. Permanganate solution decolorized. Molisch, anthrone, and $FeCl_3$ tests negative. Most stable at an acid pH (2). *Azalomycin F:* Neutral compound. White needle-shaped crystals; m.p. 125–127°C (decomposition). C = 60.41%; H = 8.57%; N = 4.33%. $C_{30}H_{50}O_{10}N_2$. Molecular weight (Berger-Akiya) 600. Molecular weight 598.72. $[\alpha]_D^{22}$ = +46 (c = 1 per cent in methanol). Light-absorption maxima at 240 and 268 mμ in methanol. Infrared absorption spectrum given in reference 2. Soluble in methanol and ethanol. Moderately soluble in 20 per cent aqueous acetone. Slightly soluble in acidic water. Insoluble in alkaline water, acetone, ethyl acetate, and chloroform. Dark brown color in Tollen reaction. Brown color in concentrated H_2SO_4. Wine-color in concentrated HCl. Positive Molisch, anthrone, Fehling, $FeCl_3$, ninhydrin, Millon, and biuret tests. Positive ninhydrin reaction after 2 minutes of hydrolysis with 5 *N* HCl. Most stable at an alkaline pH.

Biological activity: Azalomycin B: Active *in vitro* against gram-positive bacteria, including clostridia. Inactive against mycobacteria, gram-negative bacteria, and fungi. *Azalomycin F:* Active *in vitro* against gram-positive bacteria and fungi, including yeasts and *Trichomonas vaginalis*.

Toxicity: LD_{50} (mice): azalomycin B, 281 mg per kg; azalomycin F, 25.9 mg per kg intraperitoneally.

References:

1. Arai, M. J. Antibiotics (Japan) **13A:** 46–50, 1960.
2. Arai, M. J. Antibiotics (Japan) **13A:** 51–56, 1960.

Azaserine

Produced by: Streptomyces fragilis (1). One or more antibiotics in addition to azaserine are produced by this culture (12).

Method of extraction: I. Broth-filtrates flash-evaporated at <35°C. Concentrates extracted into 95 per cent ethanol and extracts filtered; chromatographed on alumina at pH 5.0 to 6.0 or pH 7 to 8 and developed with graded quantities of water in ethanol and finally water. Active eluates are concentrated, adsorbed on carbon from 1 to 2 per cent aqueous acetone or from water, and eluted with 1 to 5 per cent acetone. Active fractions are freeze dried. Crystallized from boiling 90 per cent ethanol on cooling. Recrystallized from 90 per cent methanol or 90 per cent ethanol (4).

Chemical and physical properties: O-Diazoacetyl-L-serine (5). Long light yellow-green needles; m.p. 146–162°C (decomposition). Very soluble in water. Slight solubility in cold absolute methanol, ethanol, and acetone increased by warming. Ultraviolet absorption spectrum maximum at 250.5 mμ ($E_{1cm}^{1\%}$ 1140) (pH 7.0 phosphate buffer). In 0.1 *N* NaOH, biological activity is destroyed and the ultraviolet absorption maximum shifts to 252 mμ ($E_{1cm}^{1\%}$ 1230). In 0.1 *N* HCl, N_2 is evolved, biological activity is destroyed, and the ultraviolet absorption disappears (3, 4). Infrared spectrum given in reference 4. $[\alpha]_D^{27.5}$ = −0.5° (c = 8.46 per cent in water at pH 5.18). Rotation in 2 *N* HCl changes until a constant value of $[\alpha]_D^{28}$ = +9.7° is reached. pK_a' = 8.55. Positive ninhydrin, sodium β-naphthoquinone-4-sulfonate, and ammonium silver nitrate tests (4). Crystallographic data given in reference 4. Most stable at pH 6 to 8 (4). $C_5H_7N_3O_4$. C = 34.85%; H = 4.36%; N = 24.44%; O = 37.36% (4). Structural formula (5) is given in Chapter 6. Azaserine has been synthesized, and both the DL- and D-forms prepared (6).

Biological activity: In vitro: Moderately active on gram-positive and gram-negative bacteria, mycobacteria, and fungi (2, 12). Amoebicidal to *Endamoeba histolytica* at 50 to 100 μg per ml (22). Active on *Chlorella pyrenoidosa* (27). Produces greatly elongated, multinucleate, and nonseptate filaments in *E. coli* at barely inhibitory levels and higher (11). Induces formation of active phage from lysogenic *E. coli* strain K-12 (17). The D-form has no activity on *Kloeckera brevis* (6) or other organisms (13). *In vivo:* A correlation between the activity of azaserine on *K. brevis in vitro* and its activity on tumors *in vivo* was found (2). Active on *Plasmodium lophurae* (chicks), rickettsia of epidemic typhus (eggs), meningopneumonitis virus (eggs), but not mycobacterial or other viral infections (mice) (9). *In mice:* active on 6C3HED lymphosarcoma (ascites and solid), C3H-SX lymphoma (ascites and solid) (29); moderately active on adenocarcinoma EO771, Patterson lymphosarcoma, Mecca lymphosarcoma, sarcoma 180 (ascitic and solid), Ehrlich ascites carcinoma, and Krebs-2 ascites carcinoma; slightly active on transmitted leukemia 82 and L1210 leukemia (7, 19, 28). *In rats:* moderately active on Walker carcinosarcoma 256, sarcoma R 39, Jensen sarcoma, and Murphy-Sturm lymphosarcoma; slightly active on Flexner-Jobling carcinoma (8, 19, 28). Ascitic plasma cell neoplasm of mice (70429) was initially inhibited but later developed

transmissible resistance (25). Inhibits purine synthesis in normal and tumor cells of animals and *E. coli* (20, 21). Inhibits incorporation of formate into the nucleic acids of sarcoma 180, adenocarcinoma EO771, as well as normal tissues such as the intestine and liver (mice) (8)

Toxicity: MLD (mice) 62 to 124 mg per kg intravenously (16); 25 mg per kg per day in rats produces lesions of the pancreas, liver, and kidney, as well as depletion of cellular elements in the bone marrow, reticulocytopenia, and granulocytopenia (10). Administered during the 8th to 12th day of gestation, may cause fetal resorption and teratogenic effects in rats, but does not have adverse effects on the mother even after five consecutive abortions, delay mating, or harm subsequent litters. The fetus is directly affected, not the ovaries, placenta, or pituitary (24). In chick embryos, azaserine given from the 3rd to 5th day of incubation causes skeletal abnormalities and developmental defects (23). Toxic to the canine fetus (26). In human beings, causes mouth lesions, anorexia, apathy, nausea, vomiting, and some leukopenia (14). Growth of roots (but not shoots) of cucumber, barley, and flax seedlings is inhibited by <3 μg per ml (15).

Utilization: Some beneficial results in Hodgkin's disease, chronic lymphatic leukemia, and acute leukemia in children, but probably will be used, if at all, only in combination with other drugs (14, 31).

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Azomycin

Produced by: Nocardia mesenterica (2, 6), *Streptomyces eurocidicus* (4), and a *Streptomyces* sp. resembling *S. eurocidicus*. *N. mesenterica* also produces mesenterin and Antibiotic 446. *S. eurocidicus* also produces tertiomycin and eurocidin. The *Streptomyces* sp. resembling *S. eurocidicus* also produces methymycin (9).

Synonym: Antibiotic 11A (9).

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 2.0; pH of extract adjusted to 7.0. Concentration *in vacuo*. Crude crystals form on cooling. Recrystallization from methanol or ethanol (1).

Chemical and physical properties: Acidic substance. White needle-shaped crystals (4) or color-

less prisms (7); m.p. 281–284°C (decomposition) (1, 4, 7). Soluble in aqueous NaOH and NH_4OH (yellow solutions). Slightly soluble in methanol, ethanol, propylene glycol, acetone, ethyl acetate, and butyl acetate. Almost insoluble in water, carbon disulfide, carbon tetrachloride, ether, and petroleum ether (7). Ultraviolet absorption maximum at 313 ($E^{1\%}_{1cm}$ 680) or 314 mμ ($E^{1\%}_{1cm}$ 905) in ethanol. Infrared spectrum given in reference 7. Optically inactive in methanol. Negative Pauly, Sakaguchi, $FeCl_3$, ninhydrin, biuret, Fehling, Molisch, and Millon tests. After catalytic hydrogenation, gives positive Pauly reaction. No S or halogen (1, 4, 7). At pH 2.0 to 8.0, 50 per cent of the antibiotic activity lost at 100°C in 30 minutes (4). $C_3H_3N_3O_2$ (3): C = 31.89%; H = 2.65%; N = 36.75% (7). 2-Nitro-imidazole. Structural formula given in Chapter 6. *Acetylaminoazomycin derivative:* Fine needles; m.p. 278–279°C (decomposition) (7).

Biological activity: Inhibits the growth of gram-positive and gram-negative bacteria at 3 to 25 μg per ml. Active on mycobacteria. No antifungal activity (1). Active on *Trichomonas* but not *Euglena gracilis* or *Tetrahymena geleii* (8). Inhibits ascites increase in mice with Ehrlich carcinoma but does not prolong survival time (5).

Toxicity: LD_{50} (mice) 80 mg per kg intravenously (1).

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Bacteriolytic Factors

Produced by: Various species of *Streptomyces* capable of producing enzymatic systems with the capacity to cause the lysis of dead and living bacteria. Hence they are designated as bacteriolysins. Actinomycetin, described previously, belongs to this group of compounds. Theoretically these substances should be considered as antibiotics, since they are produced by microorganisms and are able to suppress the growth of and even to kill microorganisms.

Method of extraction: The best yield of bacteriolysin is obtained on semisynthetic medium containing casein hydrolysate as a source of nitrogen. Production rate is greater at 37°C than at 30°C. The preparation can be concentrated by precipitation with $(NH_4)_2SO_4$, followed by dialysis of the dissolved precipitate.

Biological activity: Lysis of living bacteria slower than that of dead cells. Highly fibrinolytic, causing hydrolysis of extracts of M antigen from *Streptococcus pyogenes.*

Reference: 1. Pakula, R. *et al.* Acta Microbiol. Polon. **3:** 363–371, 1954.

Bamicetin

Produced by: Streptomyces plicatus (1).

Remarks: Produced simultaneously with amicetin and amicetin B (plicacetin); differs from amicetin in having one less —CH_2 group in the glycosidic moiety.

Synonym: Antibiotic D (1).

Method of extraction: Broth acidified and filtered. Filtrate (pH 5.5) concentrated *in vacuo.* Concentrate extracted in a Podbielniak extractor with 1-butanol at pH 8.6. Extract back-extracted into 0.05 *N* H_2SO_4; pH of acidic extract adjusted to 5.5. Concentration *in vacuo.* Residue extracted into butanol at pH 8.6. Addition of acetone followed by butanol-acetone (50:50) gives gelatinous precipitate (I). A water suspension of I stirred with acetone gives a granular precipitate (II), which is a mixture of amicetin and bamicetin. II suspended in dipotassium hydrogen phosphate, adjusted to pH 8.2 with HCl, water added, and mixture extracted with chloroform in a Podbielniak extractor. Chloroform-extracts contain the amicetin; the aqueous solution (III), bamicetin. III adjusted to pH 5.5 and concentrated *in vacuo.* Residue extracted with butanol at pH 8.5. Butanol concentrated. Addition of acetone, followed by acidic butanol-acetone gives the hydrochloride. Conversion to the base, followed by treatment with absolute ethanol to give the high melting point form. The ethanol-insoluble powder (IV) leached with water. Purification by dissolving IV in dilute aqueous methanol, lyophilization, and recrystallization from absolute ethanol (2, 3).

Chemical and physical properties: Dense white microcrystals; m.p. 240–241°C (decomposition). $[\alpha]_D^{23} = +123°$ (c = 0.5 per cent in 0.1 *N* HCl). Has

the same ultraviolet and infrared absorption spectrum as amicetin. Bamicetin is more water-soluble than amicetin. Can be differentiated from amicetin by X-ray diffraction pattern studies or countercurrent distribution (1-butanol-0.1 *M* phosphate buffer, pH 6.9). Anhydrous, high melting point form less soluble in water than the hydrated form. Rf value = 0.22 (1-butanol saturated with 0.05 *M* pH 7.0 phosphate buffer). Negative Bratton-Marshall and Ehrlich tests. $C_{28}H_{40}N_6O_9$: C = 55.16%; H = 6.81%; N = 13.62% (1). Acid hydrolysis yields cytimidine and the monomethylaminoglycoside (bamicetamine), $C_{13}H_{25}NO_6$ (1–3).

Biological activity: On a weight basis, has twice as much activity as amicetin against *E. coli* (1).

Toxicity: Less irritating than amicetin when administered subcutaneously to dogs (1).

References:

1. British Patent 707,332, April 14, 1954.
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Blasticidins

Produced by: Streptomyces griseochromogenes.

Method of extraction: Broth-filtrate extracted with butanol at pH 4.0. Extract shaken with sodium bicarbonate solution, then water. Solvent concentrated, then dried *in vacuo*. Residue extracted with methanol. Extract evaporated *in vacuo*. Resulting powder extracted with ether. Ether-insoluble residue named blasticidin A. Petroleum ether added to the ether precipitates crude blasticidin C. After concentration of the petroleum ether to a syrup and distillation *in vacuo*, a colorless liquid, blasticidin B, is obtained. Purification of blasticidin C by chromatography on alumina from chloroform solution, and elution with ethanol.

Chemical and physical properties: A: Light yellow powder. Soluble in water, methanol, ethanol, and water-saturated butanol. Insoluble in benzene, ether, petroleum ether, chloroform, and acetone. Ultraviolet light-absorption spectrum shows one peak at 216 mμ. *B:* Colorless liquid; b.p. 36°C at $\frac{1}{1000}$ mm Hg. Soluble in methanol, ethanol, benzene, ether, acetone, petroleum ether, and chloroform. Insoluble in water. *C:* Light reddish brown powder. Soluble in ether, acetone, and chloroform. Insoluble in water, benzene, and petroleum ether. Broth activity stable to boiling for 55 minutes at pH 5.0 to 7.0; unstable when heated above or below this range.

Biological activity: A, B, and C are moderately active on a variety of fungi, including *Piricularia oryzae*. A is most active (<1 to 100 μg per ml) (1, 2).

References:

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Blasticidin S

Produced by: Streptomyces griseochromogenes. Produced by same culture that forms blasticidins A, B, and C.

Method of extraction: Broth-filtrate treated with activated carbon at pH 4.0. Elution with 50 per cent aqueous acetone. Eluate concentrated *in vacuo* at 40°C. Oxalic acid added to the concentrate to remove the Ca^{++}. Filtrate passed through an 1R4B column to remove excess oxalate. Effluent chromatographed on IR-50 (H^+). Elution with acetone-1 *N* HCl, 1:1. Precipitation of antibiotic on addition of acetone. Purification by chromatography on alumina from a 90 per cent aqueous methanol solution. Developed with 70 to 90 per cent aqueous acetone and eluted with 50 per cent aqueous acetone. Recrystallized from aqueous acetone.

Physical and chemical properties: Free base: White needles; m.p. 235–236°C. Soluble in water and acetic acid. Insoluble in methanol, ethanol, acetone, benzene, ether, ethyl acetate, butyl acetate, chloroform, carbon tetrachloride, and methyl ethyl ketone. Negative $FeCl_3$, Fehling, Tollen, sodium nitroprusside, triphenyltetrazolium chloride, maltol, Millon, Ehrlich, Sakaguchi, Molisch, biuret, xanthoproteic, and Graf ketone tests. Positive diazo, 2,4-dinitrophenylhydrazine, ammoniacal silver nitrate, ninhydrin, and Graf aldehyde tests. $[\alpha]_D^{11} = +108.4°$ (c = 1 per cent in water). $C_{14}H_{20}O_5N_6$: C = 47.11%; H = 5.83%; N = 24.46%. Ultraviolet absorption spectrum maximum at 275 mμ ($E_{1cm}^{1\%}$ 349) in 0.1 *N* HCl or 266 to 270 mμ ($E_{1cm}^{1\%}$ 266) in 0.1 *N* NaOH. Infrared spectrum given in reference 1. Most stable at 100°C at pH 5.0 to 7.0. Less stable at pH 4.0 than pH 2.0 or pH 8.0 to 9.0. Melting points: HCl, 224–225°C (decomposition); picrate, 200–202°C (decomposition); and helianthate, 224–225°C (decomposition).

Biological activity: Active on *Pseudomonas* (5 to 50 μg per ml), slightly active on other bacteria (50 to 100 μg per ml), including mycobacteria (10 to 50 μg per ml). Active on phytopathogenic fungi (10 to 100 μg per ml), including *Piricularia oryzae* (5 to 10 μg per ml).

Toxicity: LD_{50} (mice) 2.82 mg per kg intravenously.

Reference: 1. Takeuchi, S. *et al.* J. Antibiotics (Japan) **11A:** 1–5, 1958.

Blastmycin

Produced by: Streptomyces blastmyceticus (1), and other *Streptomyces* spp. (5).

Synonyms: May be an isomer of antimycin in A_3 (5); may contain A_3 itself as the major component, with traces of A_4 (4).

Method of extraction: Filtered broth extracted with benzene. Mycelium extracted with 80 per cent aqueous acetone; acetone then evaporated. Residue extracted with benzene, and this benzene-extract combined with the benzene from the broth. After washing with phosphate buffer at pH 2.0 and evaporation *in vacuo*, an oily syrup is obtained. Syrup extracted with petroleum ether in a Soxhlet apparatus. Precipitated from the petroleum ether solution. Recrystallized from benzene-petroleum ether or ether-petroleum ether (1).

Chemical and physical properties: White needles; m.p. 167°C (1) or 168–169°C (3). Very soluble in acetone, ethyl acetate, benzene, chloroform, methyl isobutyl ketone, and carbon tetrachloride; soluble in methanol, ethanol, and ether; slightly soluble in n-hexane and cyclohexane; scarcely soluble or insoluble in petroleum ether and water. Ultraviolet absorption maxima at 225 mμ ($E^{1\%}_{1cm}$ 625) and 321 mμ ($E^{1\%}_{1cm}$ 116). Bathochromic shift on addition of base, to 222 and 245 mμ. Infrared absorption spectrum given in reference 1. $[\alpha]^{15}_{D}$ = +77.4° (c = 1 per cent in methanol). Positive $FeCl_3$, diazo, hydroxamic acid, and biuret tests; negative ninhydrin, Molisch, Fehling, Tollen, Ehrlich, and Millon tests. C = 59.81%; H = 6.89%; N = 5.34%. No S, P, halogen, or ash. $C_{26}H_{36}N_2O_9$. Acetylation gives a biologically inactive diacetyl derivative; white fibrous crystals; m.p. 146–150°C. Mild alkaline hydrolysis gives products including a neutral oil (blastmycinone), b.p. 190–191°C, and an acidic substance (blastmycic acid) (I), m.p. 141.5–142°C. Further alkaline hydrolysis of I gives formic acid and antimycic acid (structure: N-(3-aminosalicyloyl)-L-threonine), which is also a degradation product of the antimycins. Complete structure of blastmycin (1, 3) is given in Chapter 6.

Biological activity: Active on certain fungi, including *Piricularia oryzae* and *P. grisea* at 0.005 mg per ml. Also active on *Gloeosporium lacticola*, *Corticium centrifugus*, *Ophiostoma fimbriata*, *Sclerotinia hydrophilum*, but not aspergilli, penicillia, fusaria, *Elsinoe*, or *C. albicans* (1). Not active on bacteria. Inhibits anaerobic glycolysis of Ehrlich ascites tumor cells. Blocks electron transport between cytochromes B and C (2).

Toxicity: LD_{50} (mice) 1.8 mg per kg intraperitoneally (1).

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Borrelidin

Produced by: Streptomyces rochei and an unidentified *Streptomyces* sp. (1, 3).

Method of extraction: Broth-filtrate extracted with butyl acetate at pH 7. Extract evaporated to dryness. Residue purified by treatment with bentonite clay in butyl acetate, then by extraction into alkali from ether. Alkaline extract acidified and extracted with benzene. Benzene concentrated to precipitate the antibiotic. Recrystallized from benzene. Can also be adsorbed onto bentonite clay from benzene and eluted with methanol (1, 4).

Chemical and physical properties: Acidic substance; m.p. 145–146°C. Molecule may contain a site of conjugated unsaturation. Soluble in ethanol, isopropyl alcohol, and benzene. $[\alpha]^{27}_{D}$ = −28° (ethanol). Ultraviolet absorption spectrum maximum at 256 mμ ($E^{1\%}_{1cm}$ 550) (isopropyl alcohol). $C_{28}H_{43}O_6N$. Forms a crystalline methyl ester (m.p. 153–154°C) and a crystalline *p*-nitrobenzyl ester (m.p. 161°C) (1, 4).

Biological activity: Active *in vitro* and *in vivo* (mice) on *Borrelia*. Active *in vitro* on certain micrococci and *Sarcina lutea*, but not active on *Staph. aureus*, *E. coli*, or *B. subtilis*. Active on *Tetrahymena geleii*. Not active *in vivo* on other bacterial, viral, or *Treponema pallidum* infections in mice (1–4). Slightly active on carcinoma 1025 and grand epidermoid carcinoma (mice) (6).

Toxicity: LD_{50} (mice) 74.7 mg per kg subcutaneously, 39.0 mg per kg intravenously. LD_{50} (rat) 1.78 mg per kg subcutaneously (2). Poor growth in rats fed borrelidin is partially overcome by addition of niacin or tryptophan to diet (5).

References:

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2. Buck, M. *et al.* Trans. N. Y. Acad. Sci. **11:** 207–210, 1948–1949.

3. Berger, J. and Goldberg, M. W. J. Clin. Invest. **28:** 1046, 1949.
4. Jampolsky, L. M. and Goldberg, M. W. J. Clin. Invest. **28:** 1046, 1949.
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Bottromycin

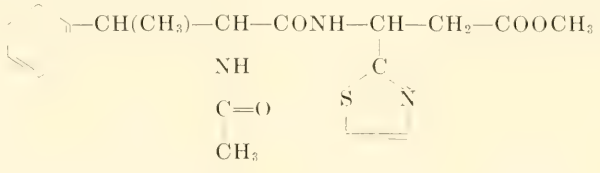
Produced by: Streptomyces bottropensis (1).

Synonym: B-Mycin (1).

Method of extraction: Broth-filtrate extracted with butyl acetate. Extract concentrated azeotropically. Concentrate washed with $NaHCO_3$ solution and water, then extracted with phosphate buffer (pH 2). Aqueous extract washed with ether to remove butyl acetate; residual ether evaporated and crude bottromycin precipitated by adjusting to pH 9. Purification by chromatography on Florisil with chloroform as solvent and developer, or by means of precipitates formed with organic acids such as salicylic, benzoic, acetic, picric, or anthranilic acids (2). Can also be adsorbed on carbon and eluted with acetone-HCl; on IRC-120 and eluted with 10 per cent NaCl; on Magnesol and eluted with benzene-methanol; or on Florisil and fractionally eluted with chloroform containing 7.5 per cent ethanol. Active fractions acidified with 0.1 *N* H_2SO_4, and organic solvent removed *in vacuo*. Precipitated from aqueous residue by adjusting pH to 9 (1).

Chemical and physical properties: Weak sulfur-containing base. Glittering, white amorphous powder; m.p. 143–147°C (decomposition). $C_{38}H_{59}N_7O_8S$: C = 58.94%; H = 7.78%; N = 12.53%; S = 3.88%. Molecular weight, about 770. pK_a' = about 6.5. Readily soluble in most organic solvents. Insoluble in hexane, cyclohexane, and petroleum ether. More soluble in ice water than water at 30°C. $[\alpha]_D^{25} = -14.2°$ (c = 0.5 per cent in 96 per cent ethanol). Ultraviolet absorption spectrum maximum at 203 mμ and a weak shoulder at 240 mμ (1, 2). Infrared data given in reference 2. Less stable at alkaline than acid pH (2). Negative ninhydrin test. One primary amino group (Van Slyke). No acetyl or N-alkyl groups. Two to three C-methyl groups and one methoxyl group. Positive bromine test. Rf = 0.94 on paper chromatography (water-saturated butanol with 2.5 per cent acetic acid). *Salicylate:* White crystals; m.p. 160–161°C. *Acetate:* Amorphous; m.p. 138–148°C (decomposition). Poorly soluble in water.

Hydrochloride: Cream-colored amorphous substance; m.p. 190–210°C (decomposition). Poorly soluble in water. Acid hydrolysis products include seven ninhydrin-positive substances, including glycine, valine, and two unidentified crystalline amino acids: (I) $C_{10}H_{13}NO_2$ (m.p. 176–177°C) and (II) $C_6H_8N_2O_2S$ (m.p. 197.5–201.5°C). Degradation with boiling acetic anhydride yields two crystalline acetyl products: (III) $C_{21}H_{34}N_4O_4$ (m.p. 165–170°C) and (IV) $C_{19}H_{23}N_3O_4S$. Compound IV is the methyl ester of an N-acetyl dipeptide composed of I and II. I and II were identified as α-amino-β-phenylbutyric acid and β-(2-thiazole)-β-alanine, respectively (3, 4). The structure of IV is:



Bottromycin contains this methyl ester grouping and is biologically inactivated on its removal (5).

Biological activity: Active on gram-positive bacteria and mycobacteria. Not active on gram-negative bacteria, except for *Hemophilus*, *Neisseria*, and *Brucella*. No cross-resistance with carbomycin or erythromycin.

References:

1. British Patent 762,736, December 5, 1956.
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Bovinocidin

Produced by: Streptomyces sp.

Synonym: β-Nitropropionic acid.

Remarks: This compound had been isolated previously from plants and fungi.

Method of extraction: Broth-filtrate adjusted to pH 2.5 and extracted with ethyl acetate. Back-extraction into pH 9.0 water. After adjustment of pH to 2.5, extraction with ethyl acetate. Ethyl acetate washed with water and evaporated to dry-

ness *in vacuo*. Residue dissolved in methyl alcohol; decolorization with charcoal. Filtrate evaporated to dryness, dissolved in anhydrous ethyl acetate, and passed through a column of alumina. Column washed with ethyl acetate, acetone, and methanol. Elution with phosphate buffer at pH 6. Adjusted to pH 3, extracted with ethyl acetate, washed with water, and concentrated *in vacuo*. A precipitate forms, which is dissolved in ether; petroleum ether added gradually. Upon standing at room temperature, colorless crystals are obtained. Recrystallization from the same ether-petroleum ether mixture.

Chemical and physical properties: Colorless crystals; m.p. 66–67°C. Soluble in water, alcohols, acetone, and ether. Moderately soluble in benzene. Insoluble in petroleum ether. C = 30.84%; H = 4.40%; N = 11.66%. $C_3H_5O_4N$. Molecular weight 119.8 (found, Rast's method, 110). Light-absorption maximum at about 270 mμ at pH 8.4. Infrared absorption spectrum given in reference 1. Bovinocidin was found to be identical to β-nitropropionic acid.

Biological activity: Weakly active against *M. tuberculosis* BCG. No activity against other mycobacteria, bacteria, and fungi tested. Biological activity not reduced by β-alanine.

Toxicity: LD_{50} (mice) 50 mg per kg intravenously.

Reference: 1. Anzai, K. and Suzuki, S. J. Antibiotics (Japan) **13A:** 133–136, 1960.

Bromtetracycline

Produced by: Certain *Streptomyces aureofaciens* (chlortetracycline-producer) mutants. Produced instead of chlortetracycline when bromide is present in a Cl-deficient medium (3).

Method of extraction: Broth-filtrate acidified to pH 2.0, mixed with Hyflo Super-Cel, and filtered. $BaCl_2 \cdot 2H_2O$ added to filtrate, pH adjusted to 8.5, and cooled to 0°C. Solids extracted with butanol at pH 2.0. Extract adjusted to pH 3.5 and concentrated *in vacuo* at <30°C. Concentrate adjusted to pH 5.0; precipitate washed with ether and dried (1). Separated from concurrently produced tetracycline by partition chromatography (butanol-chloroform over stationary Celite-aqueous HCl) (3). Purification by precipitation as HCl salt and conversion to base by bringing an aqueous solution to pH 5.2 with triethylamine and cooling. Crystallized from benzene (1).

Chemical and physical properties: Base: m.p. 170–172°C. $[\alpha]_D^{20} = -196°$ (in 0.1 *N* HCl). Ultraviolet absorption spectrum maxima (in 0.1 *N* HCl) at 227 mμ (ϵ = 17915), 260 mμ (ϵ = 17480), and 370 mμ (ϵ = 9630); or (in 0.25 *N* NaOH) at 225 mμ (ϵ = 28215), 255 mμ (ϵ = 15115), 285 mμ (ϵ = 14610), and 345 mμ (ϵ = 7221). Infrared spectrum is the same as that of chlortetracycline (1). More stable than the other tetracyclines in distilled water at 100°C for 15 minutes. $C_{22}H_{23}O_8N_2Br$ (1, 2). Structural formula given in Chapter 6. *HCl salt:* Bright yellow crystals. Browns at 218°C; decomposes at 235°C. Soluble in water to 1.36 per cent at 25°C, in dry butanol to 0.038 per cent. pK_a = 3.4, 7.4, and 9.2. $[\alpha]_D = -205°$ (0.5 per cent in 0.03 *N* aqueous HCl). Yields tetracycline on catalytic hydrogenation. Has 77 per cent of the absorption of chlortetracycline at 368 mμ (2, 3).

Biological activity: Qualitatively, antibacterial activity resembles chlortetracycline. Has 95 per cent of the activity of chlortetracycline on *Staph. aureus* on a weight basis, and 90 per cent of the activity on *E. coli* (3). Has same activity *in vivo* as other tetracyclines (2).

Toxicity: LD_{50} (HCl salt, mice) 89 mg per kg intravenously (2).

References:

1. Sensi, P. *et al.* Farmaco (Pavia) **10:** 337–345, 1955.
2. Rolland, G. *et al.* Farmaco (Pavia) **10:** 346–355, 1955.
3. Doerschuk, A. P. *et al.* J. Am. Chem. Soc. **78:** 1508–1509, 1956.

Bryamycin

Produced by: Streptomyces hawaiiensis (1, 2).

Synonym: Thiactin (2).

Method of extraction: Extracted from whole broth or broth-filtrate with methyl isobutyl ketone, butanol, or chloroform. Purification by adsorption on alumina or Florisil from chloroform; washing with water, acetone, and chloroform; and elution with a 5 per cent methanol–95 per cent chloroform mixture or aqueous t-butyl alcohol. Original methyl isobutyl ketone extract (in the cold) can be processed by "flashing" into water (distilling *in vacuo* with the addition of water until no original solvent remains) and lyophilizing, or by flashing into t-butyl alcohol. Recrystallized by flashing a concentrated chloroform solution into methyl isobutyl ketone, or by cooling a warm methyl Cellosolve or dioxane solution and addition of 10 to 50 per cent water or acetone. Forms salts with mineral acids or metals such as calcium or sodium (1, 2).

Chemical and physical properties: Amphoteric, white, crystalline, sulfur-containing polypeptide. Darkens at about 205–224°C and melts at 223–235°C. Soluble to the extent of >2 mg per ml in

formamide, chloroform, methyl Cellosolve, pyridine, glacial acetic acid, ethylene glycol monomethyl ether, 2 *N* HCl, warm butanol, warm dioxane, and hot amyl alcohol. Soluble to <2 mg per ml in the following hot solvents: acetone, benzene, amyl acetate, ethyl acetate, dimethyoxyethane, and glycol; and in cold 0.1 *N* HCl. Insoluble in water, ethanol, acetone, benzene, ethyl acetate, and dimethoxyethane (1, 2). Ultraviolet absorption spectrum maximum at 310 mμ ($E^{1\%}_{1cm}$ 125) (in 6 *N* H_2SO_4) with strong end-absorption below 250 mμ (1). Infrared spectrum given in reference 2. Paper chromatographic behavior in a variety of systems given in reference 2. $[\alpha]^{27}_{D}$ = −68.5 to −69.5° (in chloroform). Stable to boiling in aqueous solution for 1 minute. C = 51.9%; H = 5.59%; N = 16.6%; S = 9.5%. No halogen or P. Molecular weight 1600 to 1750. Acid or BaOH hydrolysis products include cystine or cysteine, threonine, α-alanine, glycine, and isoleucine. Other unidentified ninhydrin-positive substances are also present (1, 2).

Biological activity: Very active (0.002 to 0.5 μg per ml) on gram-positive bacteria and mycobacteria. Not active at 10 μg per ml on gram-negative bacteria. Inhibits *C. albicans* at 6.25 μg per ml. Active *in vivo* (mice) on *D. pneumoniae*, *Streptococcus hemolyticus*, and *S. pyogenes*, when given intraperitoneally, intramuscularly, or orally (1, 2). No cross-resistance with many commonly used antibiotics (3).

Toxicity: Mice tolerate 1 gm per kg intramuscularly, and 2 gm per kg intraperitoneally (2). LD_{50} (mice) >1 gm per kg orally (1). Material injected intramuscularly is absorbed very slowly (1).

References:

1. Cron, M. J. *et al.* Antibiotics & Chemotherapy **6:** 63–67, 1956.
2. British Patent 790,521, February 12, 1958.
3. Jones, W. F., Jr. and Finland, M. Antibiotics & Chemotherapy **8:** 387–391, 1958.

Bulging Factor

Produced by: Streptomyces sp. (2).

Method of extraction: Broth-filtrate treated with charcoal at pH 1.5 and filtered. Filtrate adjusted to pH 7.0, filtered, and retreated with charcoal. Charcoal eluted with 0.1 *N* HCl in methanol or 75 per cent acetone in water. Precipitated from methanolic eluate with ether. Acetone-eluate neutralized with IRA-400 (OH^-) and concentrated *in vacuo*. Factor precipitated as reineckate (2).

Chemical and physical properties: Basic substance. Water-soluble. Insoluble in organic solvents. Stable at acid but not alkaline pH (2).

Biological activity: Active on certain fungi, causing a pronounced bulging of the hyphal wall at intervals alternating with normal hyphal growth. Active only on fungi having chitinous cell walls (fungi imperfecti, basidiomycetes, ascomycetes, and zygomycetes), not those with cellulosic cell walls (oomycetes). Believed to act specifically on the chitin. Also active on the conidia of certain fungi, such as those of *Ophiostoma paradoxum*, which swell to 10,000 times their normal size. Not active on the bacteria or viruses tested (1, 2).

References:

1. Rombouts, J. E. Proc. 6th Intern. Congr. Microbiol. **5:** 205–207, 1953.
2. Links, J. *et al.* J. Gen. Microbiol. **17:** 596–601, 1957.

Cabicidin

Produced by: Streptomyces gougeroti (1).

Method of extraction: No data, but probably similar to other pentaene antibiotics.

Chemical and physical properties: Pentaene. Colorless or light yellow columns; m.p. 225°C. Soluble in methanol, propanol, butanol, acetone, pyridine, ethylene glycol, proplylene glycol, and glycerol. Slightly soluble in ethyl acetate and methyl Cellosolve. Insoluble in diethyl ether, petroleum ether, benzene, and water. Ultraviolet absorption spectrum maxima at 320, 339, and 354 mμ (1 per cent methanolic solution). $[\alpha]^{34}_{D}$ = −135° (methanol). Rotation changes to −87° on exposure to ultraviolet light (1).

Biological activity: Active on yeasts and fungi (1).

Reference: 1. Ogata, K. *et al.* Japanese Patent 9245, October 17, 1958.

Cacaomycetin

Produced by: Streptomyces sp. resembling *S. cacaoi*.

Method of extraction: Broth-filtrate extracted with chloroform at pH 5.0. Chromatographed on alumina and eluted with 95 per cent ethanol. Extract evaporated to dryness *in vacuo*. Residue extracted with acetone. Extract evaporated to dryness.

Chemical and physical properties: Crude yellow syrup. Slightly soluble in water. Soluble in many organic solvents. Contains four components. Very unstable at alkaline pH.

Biological activity: Active on filamentous fungi, particularly ascomycetes, but not on yeasts. Very slightly active on gram-positive bacteria and *Mycobacterium avium*.

Toxicity: MLD (mice) about 300 mg per kg subcutaneously.

Reference: 1. Wakaki, S. *et al.* J. Antibiotics (Japan) **5:** 24–49, 1952.

Caerulomycin

Produced by: Streptomyces caeruleus.

Method of extraction: Active filtrate extracted with ¼ volume of ether. Solvent evaporated to dryness *in vacuo*. Crude extract washed with cold petroleum ether, dissolved in ethanol, clarified with charcoal, and crystallized twice from ethanol. The crude extract can also be sublimed *in vacuo* at 120°C. Sublimate crystallized from ethanol.

Chemical and physical properties: Colorless needles; m.p. 175°C. Soluble in ethyl acetate, acetone, ethanol, methanol, chloroform, and ether. Sparingly soluble in benzene, petroleum ether, and water. Insoluble in sodium bicarbonate and carbonate solutions. Soluble in dilute sodium hydroxide and hydrochloric acid. Alcoholic solutions give a red color with ferric chloride. Deep red color formed when ferrous salts are added to mineral acid solutions (reaction typical of α,α'-dipyridyl). Proposed empirical formula $C_{12}H_{11}O_2N_3$, on the basis of the analysis: C = 63.09%; H = 4.99%; N = 18.48%; and molecular weights of 251 (Rast) and 215 (isothermal distillation in acetone). No terminal methyl groups (Kuhn-Roth). No acetyl groups (saponification). One methoxy group present (Zeisel) and one active hydrogen (Zerewitinoff). Amphoteric substance. pK_a = 4.38 and pK_b = 9.81. Infrared absorption spectrum given in reference 1. Light-absorption maximum at about 235 mμ with shoulders at about 285 and 295 mμ.

Biological activity: Active against yeasts and filamentous fungi at levels of 5 to 10 μg per ml. Active against gram-positive and gram-negative bacteria at levels of 10 to 100 μg per ml.

Reference: 1. Funk, A. and Divekar, T. V. Can. J. Microbiol. **5:** 317–321, 1959.

Camphomycin

Produced by: Streptomyces rutgersensis var. *castelarense* (2).

Synonym: Has properties in common with antibiotic 1-81d-1s.

Method of extraction: Agar culture extracted with ether at room temperature. Extract evaporated at <20°C *in vacuo* and residue taken up in ether. Process repeated. Residue taken up in absolute alcohol.

Chemical and physical properties: Resinous, waxy, white or white-yellow powder. Basic; m.p. 20–22°C. (A higher m.p. of 142°C (2) or 149°C (1) first reported was found (2) to be that of a decomposition product.) Soluble in ether, methyl acetate, amyl acetate, chloroform, benzene, butanol, ethanol, and methanol. Very slightly soluble in water. Ultraviolet absorption spectrum shows a progressive drop from 210 to 450 mμ, with no maxima. Positive Tollen test. Gives a precipitate with Nessler's reagent. Negative Heller, Millon, Fehling, Benedict, α-naphthol, biuret, Adamkiewicz, nitroprusside, and $FeCl_3$ tests. On heating in concentrated HCl or H_2SO_4, alcoholic solution gives a green color which turns orange, then reddish. Heating in NaOH gives a red color which clears somewhat on cooling and becomes colorless on oxidation. Reductive properties. Contains C, H, O, and N. Gives off NH_3 when boiled in NaOH. Has quinonic properties (1, 2).

Biological activity: Active mainly on gram-positive bacteria and mycobacteria. Slightly active on certain strains of *E. coli*. Active at 5 μg per ml on *Helminthosporium sativum* and *Neurospora crassa*, but not other fungi tested. Partially inactivated by thiamin and niacin (2).

Toxicity: Mice tolerate 550 mg per kg, orally or intravenously (1). Toxic to corn, oats, barley, wheat, alfalfa, cabbage, tomato, carrots, and willow twigs at 100 to 1000 ppm. Stimulates germination at 1 to 10 ppm (2).

References:

1. Cercós, A. P. Rev. arg. agron. **20:** 53–62, 1953.
2. Cercós, A. P. Rev. invest. agr. (Buenos Aires) **8:** 263–283, 1954.

Candicidin

Produced by: Streptomyces griseus (1).

Remarks: Similar to trichomycin and ascosin.

Method of extraction: I. Culture (including mycelium) adjusted to pH 2.5, stirred with Hyflo Super-Cel, and filtered. Eluted from solids with n-butanol. Extract treated with $NaHCO_3$ to remove pigmented impurities, then concentrated to dryness *in vacuo*. Residue treated with petroleum ether, taken up in water, then freeze dried. Purified by (a) treating with acetone to give Fraction C, then extracting with 95 per cent ethanol to yield Fractions A and B; (b) chromatography on a cellulose powder column from a chloroform suspension, elution with 95 per cent ethanol followed by an ethylene glycol monomethyl ether-ethanol mixture (1:1) to give Fractions A, B, and C; or (c) partition (methanol-petroleum ether-chloroform-water, 3:1:3:2) in absence of O_2 at pH 4.0.

Upper phase concentrated, then extracted with butanol at pH 7.0. Forced into a small volume of water by addition of petroleum ether to butanol (5, 7). II. Broth adjusted to pH 3.5 to 4.0 and filtered with a filter-aid. Cake extracted with water-saturated n-butanol. Extract washed with 0.333 *M* $NaHCO_3$, then with water at pH 7.0; pentane added to separate out an aqueous layer. To this aqueous layer are added aqueous washes of the remaining butanol-pentane layer, and the combined aqueous extracts are concentrated *in vacuo* and lyophilized (13).

Chemical and physical properties: Conjugated heptaene. *Crude candicidin:* Soluble in butanol, glycerol, benzyl alcohol, ethylene glycol, and ethylene glycol monomethyl ether. Partially soluble in water, ethanol, chloroform, and acetone. Insoluble in benzene, petroleum ether, carbon tetrachloride, xylene, carbon disulfide, ethylene dichloride, ether, and ethyl acetate (1). Three components were demonstrated on paper chromatography with Rf values of 0.00, 0.44, and 0.66 [methanol-0.880 (*sic*; probably specific gravity) ammonia-water, 20:1:4]. N = 1.58 to 2.16%. Nondialyzable (1, 7). *Fraction A.* Reddish brown substance. Soluble in water, ethanol, butanol, ethylene glycol, and ethylene glycol monomethyl ether. Insoluble in acetone (1). Ultraviolet absorption spectra at 340, 360, 380, and 403 mμ (ethanol) (5). C = 62.9%; H = 9.6%; N = 4.7%. At pH 7, withstands heating for 10 minutes at 60°C (1). *Fraction B:* Greenish substance. Soluble in butanol, ethylene glycol, and ethylene glycol monomethyl ether. Insoluble in water, ethanol, and acetone (1). Ultraviolet absorption spectrum maxima at 340, 362, 381, and 404 mμ (ethanol) (5). C = 57.8%; H = 9.9%; and N = 7.3% (1). *Fraction C:* Reddish brown substance. Soluble in ethanol, butanol, ethylene glycol, ethylene glycol monomethyl ether, and acetone. Insoluble in water. Ultraviolet absorption spectrum maxima at 358, 379, and 402 mμ (1). Fractions A and B are different forms of the same compound; A is the sodium salt of B. Fraction C is probably a degradation product of candicidin. Candicidin contains an aromatic component in its molecule, and is ninhydrin-positive (with two amino groups). The molecule of candicidin contains mycosamine and *p*-aminoacetophenone (14).

Biological activity: Active mainly on yeasts, but also on certain filamentous fungi, including a variety of plant pathogens. Fractions A and B differ only quantitatively in antifungal activity. Not active on bacteria, actinomycetes (including *Nocardia asteroides*), or mycobacteria. Fraction C is relatively inactive. A and B are most active at pH 7 to 8 (100 times more active than at pH 5 to 6). In tissue culture, inhibits pathogenic (yeast) stage of *Histoplasma capsulatum, Cryptococcus neoformans, Candida albicans,* and *Blastomyces dermatitidis.* Protects against wheat and *Poa pratensis* stem rust (*Puccinia graminis*) and infections by *Cronartium ribicola* (uredial stage). Some control of snapdragon rust (*Puccinia antirrhini*) and powder mildew of beans. Excellent control of bean rust (*Uromyces phaseoli*) and brown rot of peach (*Monilinia fructicola*) (1–4, 10–12, 15).

Toxicity: Crude candicidin: LD_{50} (mice) 663 mg per kg subcutaneously, 79 mg per kg intraperitoneally. Nontoxic to germination of pea seeds at 125 μg per ml or less (1). *Candicidin A:* LD_{50} (mice) 47 to 65 mg per kg intraperitoneally, 277 mg per kg subcutaneously. Necrosis at injection site (subcutaneous or intradermal). Conjuctiva of rabbits and oral mucosa (human) not irritated by a 1 per cent solution applied topically. Least inhibitory dose (tissue culture) 20 to 80 μg per ml. *Candicidin B:* LD_{50} (mice) 159 mg per kg subcutaneously, 53 mg per kg intraperitoneally (1, 2, 4).

Utilization: Vulvovaginitis (8). Intertriginous moniliasis (6).

References:

1. Lechevalier, H. *et al.* Mycologia **45:** 155–171, 1953.
2. Kligman, A. M. and Lewis, F. S. Proc. Soc. Exptl. Biol. Med. **82:** 399–404, 1953.
3. Alcorn, S. M. and Ark, P. A. Plant Disease Reptr. **38:** 705–709, 1954.
4. Hu, F. *et al.* Arch. Dermatol. Syphilol. **70:** 1–15, 1954.
5. Vining, L. C. *et al.* 8th Congr. intern. botan., Paris Vol. prelim. Sect. 24, 106–110, 1954.
6. Franks, A. G. *et al.* J. Invest. Dermatol. **23:** 75–76, 1954.
7. Vining, L. C. *et al.* Antibiotics Ann. 980–987, 1954–1955.
8. Fox, J. L. Antibiotic Med. **1:** 349–350, 1955.
9. Oroshnik, W. *et al.* Science **121:** 147–149, 1955.
10. Ark, P. A., and Alcorn, S. M. Plant Disease Reptr. **40:** 85–92, 1956.
11. Larsh, H. W. *et al.* Antibiotics Ann. 918–922, 1956–1957.
12. Muller, W. H. Am. J. Botany **45:** 183–190, 1958.
13. Siminoff, P. U. S. Patent 2,872,373, February 3, 1959.
14. Borowski, E. Personal communication.
15. Lechevalier, H. Antibiotics Ann. 614–618, 1959–1960.

Candidin

Produced by: Streptomyces viridoflavus.

Synonyms: Originally the confusing designations candidin A and candidin B referred to different salt forms of the basic candidin molecule. Candidin may exist as different salt forms, depending on the pH of isolation.

Method of extraction: Whole broth mixed with Hyflo Super-Cel, adjusted to pH 4.8 with dilute acid to precipitate the colloidally suspended candidin, and the whole filtered. Solids extracted with butanol-methanol (4:1), water added until separation into phases begins, and pH adjusted to 7.0 with NH_4OH. Organic phase concentrated *in vacuo* at low temperature until precipitate forms. A pyridine-glacial acetic acid (10:1) solution of this precipitate is heated to 50°C, warm water added, and the whole cooled. Precipitate which forms is washed with acetone and crystallized from 90 per cent aqueous dioxane (8).

Chemical and physical properties: Amphoteric, conjugated heptaene macrolide. Rosettes (from aqueous dioxane) or needles. No melting point; decomposes at >180°C. Soluble in dimethylformamide, pyridine, and dimethyl sulfoxide. Less soluble in glacial acetic acid. Slightly soluble in methanol, 70 per cent 1-propanol, water-saturated butanol, and 90 per cent dioxane. Nearly or completely insoluble in ethanol, butanol, t-butanol, dioxane, toluene, petroleum ether, sodium carbonate, and water. Ultraviolet absorption spectrum maxima at 365, 385 ($E^{1\%}_{1cm}$ 1600), and 408 mμ (alcohols). Shows a reflectance peak at 340 mμ in water suspension, a characteristic shared only by amphotericin B among the better known heptaenes. Infrared spectrum given in reference 5. $[\alpha]^{27}_{D}$ = +363°C (c = 0.3 per cent in dimethylformamide), or +205° (c = 0.3 per cent in glacial acetic acid). Positive bromine, $KMnO_4$, ninhydrin, antimony trichloride, and sulfuric acid chromophore (deep blue) tests. Negative ninhydrin (after hydrolysis), semicarbazide, Benedict, Fehling, Tollen, Schiff, 2,4-dinitrophenylhydrazine, $FeCl_3$, and pine splint tests. Can be differentiated by paper chromatography and by biological assay from the other well known heptaene antibiotics (candicidin, ascosin, trichomycin, and amphotericin B). Photo-, thermo-, and acid-labile. Most stable at neutral and alkaline pH. Stable in dry form at low temperatures under N_2. $C_{46}H_{73}O_{16}N$. Molecular weight 895. C = 60.06%; H = 9.76%; N = 1.65%. Contains four methyl groups, one primary amino group, and one carboxyl group. Acid degradation products include mycosamine. Lactone contains a series of secondary and tertiary hydroxyl groups. *N-acetylcandidin:* m.p. 159–161°C. *N-acetylcandidin, brucine salt:* m.p. 167–168°C. Hydrocandidins are biologically inactive (1, 3–5, 8, 9).

Biological activity: Active on yeasts and filamentous fungi, including plant pathogens. Completely suppresses all fungi in the fecal flora of mice dosed orally, with a concomitant 1000-fold increase in bacterial count. Active in mice with *Histoplasma capsulatum* and *C. albicans* infections. Most active on solid media at an alkaline pH (about 8.0). *N-acetylcandidin:* one-tenth as active as candidin (1, 3, 4, 6, 9).

Toxicity: LD_{50} (mice) 7 to 36 mg per kg intraperitoneally, 1.5 mg per kg intravenously, and 30 mg per kg subcutaneously (with necrosis at injection site). Splenic atrophy observed after oral administration of 100 mg per kg to mice (1, 3).

References:

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2. Taber, W. and Vining, L. C. Bacteriol. Proc. 86, 1954.
3. Vining, L. C. *et al.* Antibiotics Ann. 980–987, 1954–1955.
4. Oroshnik, W. *et al.* Science **121:** 147–149, 1955.
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6. Muller, W. H. Am. J. Botany **45:** 183–190, 1958.
7. Solotorovsky, M. *et al.* Antibiotics & Chemotherapy **8:** 304–371, 1958.
8. Borowski, E. Personal communication, 1959.
9. Lechevalier, H. unpublished data.

Candimycin

Produced by: Streptomyces echimensis (1).

Synonyms: Closely related to ascosin and candicidin (1).

Method of extraction: Extracted from broth and mycelium; purification by chromatography (1).

Chemical and physical properties: Yellow. Soluble in aqueous acetone, alcohol, glycol, and dilute NaOH. Less soluble in water. Insoluble in ether, benzol, acetone, and chloroform. Contains no S or halogen. C = 57.17%; H = 8.18%; N = 1.70%. Negative $FeCl_3$, ninhydrin, Molisch, Fehling (cold), and Sakaguchi tests. Silver mirror test positive in hot state. Addition of H_2SO_4 changes the powder to a blue, then to a violet color. Ultraviolet maxima (methanol) at 362, 382, and 406 mμ. Stable to acid and alkali pH (1).

Biological activity: Very active on fungi, yeasts, and *Trichomonas vaginalis* (1). Active in rats on Yoshida sarcoma cells, but does not prolong survival time. Not active on Ehrlich carcinoma (mice) (2).

Toxicity: LD_{50} 5 mg per kg subcutaneously; animal not given.

References:

1. Shibata, M. *et al.* J. Antibiotics (Japan) **7B:** 168, 1954.
2. Aramaki, Y. *et al.* Ann. Rept. Takeda Research Lab. **14:** 60–91, 1955.

Carbomycin

Produced by: Streptomyces halstedii (1, 26) (also produces carbomycin B), *S. hygroscopicus* (8), and *S. albireticuli* (21).

Synonyms: Magnamycin; carbomycin A, antibiotic M 4209 (9).

Methods of extraction: I. Broth-filtrate extracted with benzene, chloroform, amyl acetate, trichloroethylene, butanol, or ether at pH 6.6 or higher. Solvent: (A) Evaporated to dryness. Residue yields crystals when taken up in a small amount of isopropanol. Recrystallized from aqueous acetone or ethanol. (B) Solvent distilled off in the presence of water. Watery residual solution: (a) freeze dried and recrystallized from benzene-petroleum ether or aqueous methanol; (b) chromatographed on silica gel from 10 per cent acetone in benzene and developed with acetone-benzene mixtures containing gradually increasing amounts of acetone, the principal fraction being in the 30 per cent acetone in benzene; solvent evaporated off; residue crystallized from 70 per cent hot aqueous isopropanol on addition of water; or (c) extracted with neutral benzene, extract concentrated; concentrate back-extracted into water at pH 2.0 to 2.5; aqueous extract neutralized to give free base (8, 9, 22). II. Broth-filtrate extracted with methyl isobutyl ketone at pH 3.0. Extract concentrated *in vacuo* and extracted with dilute sulfuric acid (pH 2.0). Aqueous extract washed with benzene, adjusted to pH 6.5, and extracted with ether repeatedly. Ether evaporated to dryness. Residue triturated in ethanol. Recrystallized from methanol-water (10). III. Carbomycin forms complexes with such aromatic solvents as benzene, mesitylene, benzyl chloride, toluene, ethyl benzene, naphthalene, chlorobenzene, and toluene. Such complexes may be precipitated by addition of hexane to a sufficiently pure solution of the complex or by concentration *in vacuo*. The complex may be cleaved to give the free base as shown previously (24). These complexes may also be separated as solids from the crude broth-filtrate if sufficient complexing agent is added at pH 8.5 to 9.5. Cleavage is obtained with acid, or the complex extracted into acetone or methanol and the extract concentrated to remove the solvent. Base precipitates on addition of water to the residue (25).

Chemical and physical properties: Weakly basic (26). Macrolide (28). *Base:* Various crystal forms have been reported. Prisms; m.p. 207–209°C (open capillary in oil bath) or 220–222°C (hot stage) (22); slender white blunt-ended needles; m.p. 199.5–200.5°C (1); or rectangular plates or laths; softens at 208–210°C; m.p. 210–216°C (decomposition) (9, 10). Very soluble in chloroform, ethyl acetate, glacial acetic acid, 0.05 *N* HCl, amyl acetate, butanol, benzene, and acetone. Less soluble in methanol, ethanol (25 mg per ml), ethyl ether, and isopropanol (6.2 mg per ml). Insoluble in hot or cold water, aqueous alkali, and petroleum ether 9, 22). Ultraviolet spectrum maxima at 238 mμ ($E^{1\%}_{1cm}$ 185) and 327 mμ ($E^{1\%}_{1cm}$ 0.9) in absolute ethanol (10) or at 241 mμ ($E^{1\%}_{1cm}$ 158) in 2 per cent Na_2HPO_4 (22). Infrared spectrum given in references 10, 22, and 26. $[\alpha]^{22}_D = -53° \pm 3°$ (c = 0.3 per cent in 0.05 *N* acetic acid), or −56.1° (c = 1 per cent in chloroform) (10, 22). Positive 2,4-dinitrophenylhydrazine, Bayer permanganate, bromine, and ceric nitrate tests. Positive (10) or negative (22) Fehling and Tollen tests. Violet color with Schiff's fuchsin reagent (9). Violet color in 4 *N* HCl (10) or red-violet with strong acid (5). Intense yellow color in methanolic KOH (9). Negative ninhydrin, Van Slyke, boric acid, and $FeCl_3$ tests (10). Water-insoluble precipitate with trichloroacetic acid (9). No precipitate with silver nitrate or mercuric chloride (22). Rf values in various systems given in references 8 and 22. Most stable in aqueous solutions at pH 5 to 7; less stable at pH 3 or 9. Crystals stable for several months in the dark at room temperature (10). C = 59.89%; H = 7.96%; N = 1.78%; C—CH_3 = 10.58%; N—$(CH_3)_2$ = 2.94%; OCH_3 = 3.75% (12). No S, P, or halogen (5). Molecular weight 810 to 866 (9, 12, 26). $C_{42}H_{67}O_{16}N$. Structural formula is given in Chapter 6. Mild acid hydrolysis yields, among other products, the 4-isovaleryl methyl glycoside of the sugar *mycarose*. The glycoside is an oily neutral substance; b.p 116°C (1.1 mm), n^{25}_D 1.4493; $[\alpha]^{25}_D = -10.7°$ (c = 9 per cent in chloroform). *Mycarose* itself is a crystalline solid; m.p. 128–129°C. $[\alpha]^{25}_D = -31.1°$ (c = 4 per cent in water) (11). Strong acid hydrolysis yields *mycaminose*, $C_8H_{17}NO_4$. Hydrochloride: m.p. 115–116°C. $[\alpha]^{25}_D = +31°$ (c = 1 per cent in water) (20). Methanolysis removes mycarose,

yielding the unstable crystalline base *carimbose*, $C_{30}H_{47}O_{12}N$ (28, 29); m.p. 188–189°C. $[\alpha]_D^{25} = -24°$ (c = 1 per cent in ethanol). Ultraviolet absorption spectrum maximum at 240 mμ ($E_{1cm}^{1\%}$ 196) and a weak band at 285 mμ ($E_{1cm}^{1\%}$ 1.4) in phosphate buffer at pH 6.0 (9, 12). Hydrogenation yields a biologically active tetrahydroproduct: long colorless needles, softens at 116°C, liquifies at 118–122°C. $[\alpha]_D^{25} = -53°$ (c = 0.5 per cent in ethanol). Ultraviolet absorption spectrum maximum at 330 mμ. Carbomycin forms the following salts: *Hydrochloride:* Amorphous substance (22); m.p. 157–160°C (decomposition), softens at 155°C (9, 22) or 149–150°C (12). Soluble in water, methanol, and ethanol. Insoluble in ethyl ether and hexane. Same $[\alpha]°$ as carbomycin base. *Sulfate:* Crystalline; m.p. 163–164°C (decomposition); shrinks at 158°C (9). *Diacetate:* m.p. 149–151°C. *Pentaacetate:* m.p. 134–135°C. *Thiosemicarbazone:* m.p. 170–173°C. Ultraviolet maxima at 230 mμ ($E_{1cm}^{1\%}$ 218) and 268 mμ ($E_{1cm}^{1\%}$ 268) (9, 12). *Oxime:* Crystalline; m.p. 198–199°C (10). *Periodate:* White crystals; m.p. 134–135°C (decomposition) (10).

Biological activity: In vitro: Active on gram-positive bacteria but not gram-negative bacteria, except *Hemophilus.* Active on *Actinomyces israelii* and mycobacteria but not on *Nocardia asteroides.* Not active on fungi. Active on pleuropneumonia-like organisms (PPLO) (1, 2, 6, 10). Moderately active on *Endamoeba histolytica, Trypanosoma cruzi, Leishmania donovani;* less active on *T. rhodesiense, Trichmonas vaginalis,* and *T. foetus* (5). Cross-resistance with erythromycin, oleandomycin, spiramycin, and streptogramin (23), resistance developing slowly in a stepwise pattern (1). Activity unaffected by serum (10). The highest concentration permitting epithelial cell migration in tissue culture is 190 μg per ml (30). *In vivo:* Active (in mice) on infections caused by *Streptococcus pyogenes, D. pneumoniae, Staph. aureus,* and *Pasteurella multocida* (4, 16). Antitoxoplasmic activity (rabbits) (17). Active on PPLO causing the air sac disease *in ovo* and in adult chickens (10). Active on the following rickettsiae in eggs and a variety of experimental animals: *Rickettsia prowazekii, R. mooseri, R. typhi, R. akari, R. tsutsugamushi, R. rickettsii, R. conorii, Coxiella burnetii,* and North Queensland tick typhus. Also active on the organisms causing psittacosis, lymphogranuloma venereum, human and feline pneumonitis, bovine encephalomyelitis, ornithosis, canine distemper, meningopneumonitis, and bluetongue (Sonora strain). Such rickettsiae and psittacosis-like forms can be recovered from surviving treated animals, indicating a static effect of the drug. No activity on herpes simplex, rabies, vaccinia, or poliomyelitis type II (5, 7, 8, 10, 15, 18, 22). Prolongs survival time in mice infected with *Clostridium perfringens* and *C. histolyticum* (31). Anthelmintic activity against *Aspicularis tetraptera* and *Syphacia obvelata* in mice (8). Increases growth rate of poultry and swine (1, 19). Controls *Rhizoctonia* infection of lettuce (13). Causes elongation of wheat roots in water solution (3).

Toxicity: LD_{50} (mice) 550 mg per kg intravenously, 900 to 1000 mg per kg intramuscularly, 2950 mg per kg subcutaneously, and 550 mg per kg orally (1). LD_{50} (rats) 700 mg per kg intravenously, and >5000 mg per kg orally (10). Highly toxic to guinea pigs (8). Eggs tolerate 10 mg per embryo (15). Minimal dose causing inhibition of mitosis in HeLa cells is 62.5 μg per ml (27); 1 to 10 μg per ml has no effect on human spermatozoa (5).

Utilization: Infections caused by gram-positive organisms, especially staphylococci resistant to other antibiotics. Amoebiasis. Veterinary medicine. Less effective than penicillin (5, 14, 18).

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Carbomycin B

Produced by: Streptomyces halstedii. This culture also produces carbomycin (1).

Synonym: Magnamycin B.

Method of extraction: Methanol-water mother liquors from carbomycin recrystallizations are diluted with a large volume of water to give a precipitate. Dried precipitate taken up in anhydrous ethanol and stirred at room temperature for 8 hours. Carbomycin, crystallized by this procedure, filtered off. Filtrate evaporated, dissolved in acetone, and water added to turbidity. Carbomycin B separated out overnight. Recrystallized from a 5:1 acetone-water mixture and anhydrous acetone (1).

Chemical and physical properties: Probably is an $\alpha,\beta,\gamma,\delta$-unsaturated ketone. Weakly basic substance. May be isomeric with carbomycin. Colorless anisotropic plates, frequently hexagonal; m.p. 140–144°C (decomposition); softens at 138°C. Soluble at 20–25°C in methanol to 1.4 gm per ml; ethanol 0.45 gm per ml; acetone 0.25 gm per ml; benzene 0.15 gm per ml; ether 0.03 gm per ml; and water, 0.1 to 0.2 mg per ml (1, 3). Ultraviolet absorption spectrum maximum at 278 mμ ($E^{1\%}_{1cm}$ 276) (E = 25,000). Infrared spectrum given in reference 1. $[\alpha]_D^{25} = -35°$ (c = 1 per cent in chloroform). Buffered aqueous solutions (pH 5.0) have a half-life of >3 months. Less stable at pH 3 or 10. C = 60.55%; H = 8.42%; N = 1.78%; O—CH_3 = 4.01%; N—CH_3 = 3.10%. Equivalent weight 870. pK_b = 7.56. $C_{41\text{-}42}H_{67\text{-}69}NO_{15\text{-}16}$. Hydrolysis in methanolic HCl yields, like carbomycin, methyl mycarose isovalerate. Vigorous acid hydrolysis yields, again like carbomycin, mycaminose. Other evidence indicates that carbomycin B may differ from carbomycin in more than one double position, and may contain one oxygen atom less (1). *Hydrochloride:* White crystals. More soluble in water than the base, but less so than carbomycin A hydrochloride (2).

Biological activity: Resembles carbomycin (1). Stimulates rat growth without concomitant fecal microflora changes, when administered intraperitoneally, but has no growth-stimulatory effect and produces marked changes in intestinal microflora when given orally (2).

Toxicity: LD_{50} (mice) 300 mg per kg intravenously. Other routes are comparable to carbomycin (1).

References:

1. Hochstein, F. A. and Murai, K. J. Am. Chem. Soc. **76:** 5080–5083, 1954.
2. Dick, E. C. and Johansson, K. R. Antibiotics & Chemotherapy **7:** 349–358, 1957.
3. Tanner, F. W. *et al.* U. S. Patent 2,785,104, March 12, 1957.

Carcinomycin

Produced by: Streptomyces carcinomycicus, Streptomyces sp. (4), *S. gannmycicus* (2), and *S. ganmycicus* (3).

Synonyms: Gannmycin; carzinomycin (4).

Method of extraction: Precipitated from broth-filtrate with 50 per cent $ZnCl_2$. Precipitate extracted with Na_2HPO_4 at 25°C. $Zn_3(PO_4)_2$ filtered off and supernatant dialyzed against running water at 0°C for 48 hours. One per cent NaCl is added and the antibiotic reprecipitated by addition of acetone. Precipitate taken up in water and freeze dried. Cannot be extracted with organic solvents.

Chemical and physical properties: Dark brown powder.

Biological activity: Active against Ehrlich carcinoma (ascites form) in mice. No activity on bacteria or fungi.

Toxicity: Mice tolerate 500 mg per kg intraven-

ously, intraperitoneally, intramuscularly, and subcutaneously.

References:

1. Hosoya, S. Ann. Meeting Japan. Chemotherapy Soc. **3:** 128–131, 1955.
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4. Umezawa, H. Giorn. microbiol. **2:** 160–193, 1956.

Cardicin

Produced by: Nocardia sp.

Method of extraction: Mycelium extracted with hot methanol; or acidified broth extracted with butanol. Methanol concentrated *in vacuo*, and cardicin precipitated on addition of ether. I. Dissolved in dilute NH_4OH and reprecipitated on acidification. II. Purification by extraction of an acidified aqueous solution with butanol. Butanol washed with $NaHCO_3$ and water, and step II repeated.

Chemical and physical properties: Stable. Acidic form insoluble in water, saline solution, ether, and acetone; soluble in methanol, ethanol, and hydrous butanol. Sodium salt insoluble in ether and butanol; soluble in water, methanol, ethanol, and large volumes of hydrous butanol.

Biological activity: Active *in vitro* against gram-positive bacteria, fungi, and the following phages: staphylophage, streptophage, *Bacillus cereus* phage, *B. megaterium* phages, coliphage, and enterococcus phage. Active in eggs on PR 8, Lee, and FM-1 strains of influenza virus.

Toxicity: 93 μg injected intraperitoneally or subcutaneously kills mice in 2 days.

Reference: 1. Machlowitz, R. A. *et al.* Antibiotics & Chemotherapy **3:** 966–970, 1953.

Caryomycin

Produced by: Streptomyces filamentosus (1, 2).

Method of extraction: Extracted from broth-filtrate with butyl or ethyl acetate. Extracted from mycelium with alcohols, benzene, or petroleum ether (2).

Biological activity: Active on gram-positive bacteria, including mycobacteria. Less active on gram-negative bacteria. Active on Yoshida sarcoma (rats), increasing survival time and producing some cures (1, 2).

References:

1. Okami, Y. *et al.* J. Antibiotics (Japan) **6A:** 153–157, 1953.
2. Yamamoto, T. and Umezawa, H. Japanese Patent 396, January 26, 1955.

Carzinocidin

Produced by: Streptomyces kitasawaensis (2). This culture also produces actinomycin A (3).

Method of extraction: I. Formation of a precipitate by adjusting the broth-filtrate to pH 5.0 and adding 5 per cent by volume of a 20 per cent $ZnCl_2$ solution. Precipitate extracted with 1 per cent Na_2HPO_4 solution. To this solution at pH 7.0, 80 per cent saturated ammonium sulfate is added. A precipitate forms, which is dissolved in water and purified by dialysis. Addition of acetone forms a precipitate of crude brown-black carzinocidin powder (1). II. Broth-filtrate adsorbed on carbon at pH 2.4, and eluted with water at pH 8.0. Eluate concentrated at pH 4.4 and freeze dried. Purified by extracting an aqueous solution with butanol at pH 6.5; extract evaporated *in vacuo* (3).

Chemical and physical properties: Polypeptide containing cystine, lysine, glycine, and glutamic acid. No crystals form; no definite melting point. Soluble in alkaline water. Slightly soluble in 20 per cent acetone (pH 8.0), 20 per cent methanol, 20 per cent ethanol, 20 per cent pyridine, and butanol-saturated alkaline water. Insoluble in acidic water. No specific ultraviolet absorption. Positive xanthoproteic and Pauly reactions. "Pseudopositive" Millon reaction. Negative biuret, Adamkiewicz, Liebermann, H_2SO_4, ninhydrin, diphenylamine, cysteine, nitroprusside, Folin, anthrone, Sakaguchi, and $FeCl_3$ reactions. Unstable to heat, especially at alkaline pH. C = 37.2%; H = 6.1%; N = 12.2%; S = 3.5%. Molecular weight >6000 (3).

Biological activity: Slight activity against *C. albicans*, *Torula utilis*, and *Sacch. cerevisiae*. Active in mice against Ehrlich carcinoma, mainly against the subcutaneous solid tumor form. Slight activity against Yoshida sarcoma (1, 3).

Toxicity: LD_{50} (mice) 4.7 mg per kg intravenously, 43.5 mg per kg intraperitoneally, 20 mg per kg subcutaneously. Cystine and methionine do not decrease the toxicity of this substance in mice (1, 3).

References:

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Carzinophilin A

Produced by: Streptomyces sahachiroi (1, 7).

Synonym: Carzinophilin (3).

Method of extraction: Extraction of culture-filtrate with organic solvents such as butyl acetate, benzene, or chloroform at pH 6.0 to 7.0. Back-extraction in water at pH 9.0 to 10.0. Aqueous extract either (a) freeze dried; (b) precipitated by addition of $(NH_4)_2SO_4$, and precipitate purified by extraction into acetone or butanol; or (c) chromatographed on alumina at pH 5 to 8 and eluted with alkaline aqueous acetone, pH 7 to 10 (1, 7). Broth-filtrate stirred with diatomaceous earth or similar adsorbent at pH 6.5, and eluted with methanol at pH 7 to 10. Eluate concentrated *in vacuo* at about pH 7.0. Concentrate extracted with organic solvents at pH 5 to 8, then back-extracted into water at pH 7 to 10 and freeze dried (7).

Chemical and physical properties: Acidic substance. Colorless needles. Darkens above 205°C; m.p. 217–222°C (decomposition). Soluble in acetone, chloroform, ethyl and butyl acetate, benzene, dioxane, and aqueous alkali. Slightly soluble or insoluble in water, methanol, ethanol, ether, carbon tetrachloride, and petroleum ether $[\alpha]_D^{28} = +57.8°$ (chloroform). Ultraviolet absorption spectrum maxima: 218, 250, and 283 mμ (2 per cent $NaHCO_3$ or methanol); 230 mμ ($E_{1cm}^{1\%}$ 940) and 283 mμ ($E_{1cm}^{1\%}$ 460) (0.1 *N* NaOH); 219 and 252 mμ (ether); 210 and 330 mμ (acetone); 270 mμ ($CHCl_3$); 242 and 378 mμ (CS_2). Infrared data given in reference 2. Positive bromine, xanthoproteic, diphenylamine, 2,4-dinitrophenylhydrazine, and sodium nitroprusside tests. Gives the following reactions: Bayers test ($KMnO_4$ in NaOH, green); ninhydrin (yellow), and anthrone (yellow). Negative Molisch, Sakaguchi, Fehling, Benedict, $FeCl_3$, and Tollen reactions. Forms salts with alkalies. Biological activity destroyed by thiourea, cysteine, methionine, hydroquinone, vitamin B_{12}, H_2O_2, and ultraviolet light. Thermostable in dry state; unstable in aqueous solutions. C = 59.78%; H = 5.17%; N = 6.93%. Molecular weight 800 to 1200. Alkaline decomposition products include two crystalline substances: I. m. p. 78–79°C; colorless needles; $C_{14}H_{13-14}O_3$. II. m. p. 177–180°C; colorless granular crystals; $C_{12-13}H_{12-13}O_3$ (1, 3, 4, 7).

Biological activity: Active *in vitro* against gram-positive bacteria (*Sarcina lutea*) and mycobacteria. Limited activity against gram-negative bacteria, except members of the genus *Brucella* and *K. pneumoniae* (1). Active on *Nocardia asteroides* at 10 μg per ml but not on protozoa (7). Not active on fungi (1). Pure substance has about 20 times more activity on Yoshida sarcoma than crude material (3). Active on ascitic forms of Ehrlich carcinoma, hepatoma 7974, sarcoma 180, and Krebs 2 carcinoma (1, 6). Certain concentrations stimulate root tip growth of *Allium cepa*. A very slight reduction in the number of mitotic figures was noted at the highest concentration used (8). Kills HeLa cells at 0.031 μg per ml (5).

Toxicity: LD_{50} (mice) 150 μg per kg intravenously (4). Subcutaneous or intramuscular administration causes induration, necrosis, and ulceration at site of injection. Leukopenia and urobilin in urine also noted (2).

Utilization: Some effects on neoplastic disease (2, 7).

References:

1. Hata, T. *et al.* J. Antibiotics (Japan) **7A:** 107–112, 1954.
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7. British Patent 777,287, June 19, 1957.
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Catenulin

Produced by: Streptomyces sp.

Synonyms: Closely related to hydroxymycin and paromomycin.

Method of extraction: Adsorption on carbon or precipitation as the salt (I) of eriochrome violet. A methanolic solution of I treated with triethylamine sulfate gives crude catenulin sulfate. Can be crystallized as the helianthate or the *p*(*p'*-hydroxyphenylazobenzene) sulfonate salt from hot water.

Chemical and physical properties: Sulfate: Insoluble in methanol. End-absorption of ultraviolet light. Infrared spectrum said to be characteristic of a polypeptide. $[\alpha]_D^{25} = +51.9°$ (c = 1 per cent in water). C = 31.45%; H = 6.15%; N = 7.92%; SO_4 = 28.12%. All the nitrogen is basic. Prolonged acid hydrolysis yields a product tentatively identified as neamine by paper chromatography. Differentiated from neomycins A and B and the streptomycins by paper chromatography. Stable in aqueous solution from pH 1.5 to 10.0.

Biological activity: Active against mycobacteria, *K. pneumoniae*, and *B. subtilis*. Cross-resistance with neomycin and viomycin, but quantitatively different from neomycin (3).

Toxicity: LD_{50} (mice) 125 mg per kg intravenously. Causes neurotoxicity in cats. Toxic to duckweed (*Lemna minor*) at 1 ppm (2). Causes wheat root tip elongation in tap water solution at 0.1 ppm (4).

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2. Nickell, L. G. and Finlay, A. C. J. Agr. Food Chem. **2:** 178–182, 1954.
3. Szybalski, W. and Bryson, V. Am. Rev. Tuberc. **69:** 267–269, 1954.
4. Barton, L. V. and MacNab, J. Contrib. Boyce Thompson Inst. **17:** 419, 1954.

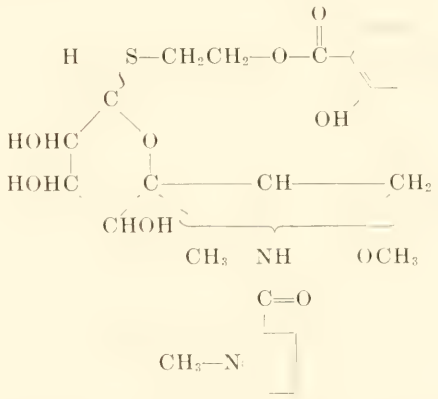
Celesticetin

Produced by: Streptomyces caelestis (3).

Synonym: Antibiotic D 52 (3).

Method of extraction: Extraction of the filtered broth with methylene chloride at pH 7.8. Concentration of the extract. Addition of petroleum ether to the concentrate precipitates crude celesticetin as a tan solid. Oxalic acid or salicylic acid added to the methanolic solutions of celesticetin. The oxalate or salicylate formed is purified by recrystallization. Reconversion of the salts to the amorphous free base accomplished by extracting their aqueous solutions at pH 7.5 with methylene chloride. Also can be extracted from broth with ethyl or amyl acetate, or butanol. Extract concentrated. Concentrate added to hexane to give amorphous celesticetin. Can also be precipitated or isolated from the organic extract of broth as the salt of an acid. Purification or initial extraction can also be accomplished by adsorption on alumina, silica gel, activated clays, or acetic acid-treated charcoal. Elution with a polar organic solvent in which celesticetin is soluble (3).

Chemical and physical properties: Amphoteric, colorless, amorphous substance. pK_a of basic group, 7.7; pK_a of acidic group, 9.8. Soluble in acidic and strongly basic aqueous solutions. Insoluble in water between pH 7 and 10. Soluble in polar solvents, but insoluble in ether or light hydrocarbons. Stable between pH 5 and 7 for 60 days or longer at 24°C. $[\alpha]_D^{24}$ for the free base = +126.6° (c = 0.5 per cent in chloroform). Maximal ultraviolet light absorption in 0.01 *N* alcoholic potassium hydroxide at 248 and 341 mμ; in 0.01 *N* alcoholic sulfuric acid at 240 and 310 mμ. Positive $FeCl_3$, Molisch, and Ekkert tests. Formation of white precipitates with bromine water, Millon's reagent, and mercuric chloride. No precipitation with silver nitrate or lead acetate. Negative Benedict, ninhydrin, and iodoform tests. C = 54.87%; H = 6.75%; N = 5.30%; S = 6.02%. Empirical formula: $C_{24}H_{36}N_2O_9S$. *Hydrochloride:* White semicrystalline powder. $[\alpha]_D^{21}$ = +96.7° (c = 0.5 per cent in water). *Oxalate:* White needles; m.p. 149–154°C. $[\alpha]_D^{24}$ = +106.4° (c = 0.5 per cent in water). Indefinite benzenesulfonyl chloride test. Negative nitroprusside, $FeCl_3$ (brown), and bromine in CCl_4 tests. Positive NaN_3-I_2 test (for C—CH or C=S). *Salicylate:* Crystals; m.p. 136–138°C. $[\alpha]_D^{24}$ = +90.2° (c = 0.5 per cent in water). All salts have essentially the same ultraviolet spectrum as the base (3). Mild alkaline hydrolysis yields salicylic acid and a basic product, $C_{17}H_{32}N_2O_7S$, called desalicetin. Acid hydrolysis yields, among other products, L-hygric acid and a reducing amino sugar, $C_9H_{19}HO_6$, and celestose. Partial structure of celesticetin (2):



Biological activity: Active *in vitro* against gram-positive bacteria at the level of 0.19 to 12.5 μg per ml. Not active against gram-negative bacteria or fungi. Active *in vivo* against experimental *Streptococcus hemolyticus* and *Staph. aureus* infections. Inactive *in vivo* against *M. tuberculosis* H37Rv and Newcastle and influenza viruses. Reported to enhance the growth of animals and poultry. Active on plant diseases, including fire blight of apple and pear trees, bacterial spot of tomatoes, walnut blight, halo blight of beans, turf diseases, mint rust, and cherry leaf spot. Active *in vitro* on *Nocardia asteroides* (3).

Toxicity: LD_{50} (mice) 167 mg per kg (free base); 233 mg per kg (oxalate) intraperitoneally (3).

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2. Hinman, J. W. and Hoeksema, H. Abstr. 129th Meeting Am. Chem. Soc. 17M–18M, 1956.
3. British Patent 768,971, February 27, 1957.

Cellocidin

Produced by: Streptomyces chibaensis (1).

Method of extraction: Broth-filtrate stirred with activated carbon at pH 6.4 to 6.8. Elution with 80 per cent aqueous methanol. Concentration of eluate *in vacuo* precipitates the antibiotic. Recrystallization from hot aqueous methanol (1).

Chemical and physical properties: Acetylenedicarboxamide (2). Formula given in Chapter 6. Melts at 216–218°C (decomposition). Slightly soluble in water, methanol, ethanol, and acetone. Insoluble in other organic solvents. Relatively stable at acid pH and neutrality. Unstable at alkaline pH, decomposing with evolution of ammonia when boiled. Ultraviolet absorption spectrum maximum at 299 mμ ($E^{1\%}_{1cm}$ 290) in 0.1 *N* NaOH) (1). Infrared spectrum given in reference 1. Catalytic reduction product is succinamide. Major acid hydrolysis product is $C_4H_3O_4Cl$ (chlorofumaric acid) (2).

Biological activity: Active on *Micrococcus flavus* and *E. coli* at 10 μg per ml, *B. subtilis* at 20 μg per ml, and *Staph. aureus* at 200 μg per ml. Active on *M. tuberculosis* BCG at 3 μg per ml. Active *in vitro* on N.F. mouse sarcoma but not *in vivo* on Ehrlich ascites carcinoma (1).

Toxicity: LD_{50} (mice) 11 mg per kg intravenously (1).

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1. Suzuki, S. *et al.* J. Antibiotics (Japan) **11A:** 81–83, 1958.
2. Suzuki, S. and Okuma, K. J. Antibiotics (Japan) **11A:** 84–86, 1958.

Cellostatin

Produced by: Streptomyces cellostaticus (1).

Synonym: Similar to blasticidin S.

Method of extraction: Broth-filtrate passed through an IRC-50 column (H^+) and eluted with 80 per cent aqueous acetone (I), followed by 80 per cent acetone containing 0.2 *N* HCl (II). Fraction I contains an unrelated anti-*Sarcina* antibiotic. Fraction II is adjusted to pH 6.0, concentrated *in vacuo*, and dried. Residue extracted with anhydrous methanol, filtered, and dried *in vacuo*. Final precipitation from acidic methanol with acetone. Purified by chromatography on alumina and Darco G-60 (1).

Chemical and physical properties: Basic substance. Very soluble in water; slightly soluble in methanol; insoluble in ethanol, butanol, acetone, ether, and other organic solvents. Forms a picrate, reineckate, and phosphotungstate. Stable at acid and neutral pH. Ultraviolet absorption spectrum maximum at 265 mμ ($E^{1\%}_{1cm}$ 115) (distilled water). Infrared spectrum given in reference 1. Positive ninhydrin, Sakaguchi, and Molisch tests. Negative biuret, Millon, Fehling, maltol, $FeCl_3$, and Elson-Morgan tests. C = 22.10% (*sic*; probably 52.10%); H = 4.52%; N = 13.15%; O = 27.77%. *Sulfate:* Colorless platelets. *Reineckate:* Crystalline substance; m.p. 156–166°C (1).

Biological activity: Very slightly active (50 to 100 μg per ml) on certain gram-positive and gram-negative bacteria, including *Micrococcus citreus*, *B. anthracis*, *Sarcina lutea*, *Salmonella*, *Ps. aeruginosa*, *Pr. vulgaris*, and *Clostridium botulinum*, but not *Staph. aureus*, *E. coli*, or *B. subtilis*. Slightly more active (6.3 to 100 μg per ml) on yeasts and fungi. Static action on *Trichomonas vaginalis* at 100 μg per ml (1). Active on Ehrlich ascites carcinoma (1, 2).

Toxicity: LD_{50} (mice) about 15 mg per kg intraperitoneally (1).

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1. Hamada, S. Tôhoku J. Exptl. Med. **67:** 173–179, 1958.
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Cephalomycin

Produced by: Streptomyces tanashiensis var. *cephalomyceticus.*

Method of extraction: Adsorption on IRC-50 or XE-64 resins (H^+). Resins washed with water. Elution with aqueous ammonia at pH 9.0. Precipitation of eluate at pH 3.6. Precipitate washed with cold 2 per cent aqueous acetic acid, redissolved in water at pH 9.0, and centrifuged. The supernatant adjusted to pH 5.1. The resulting precipitate is purified further by repeating the last series of operations.

Chemical and physical properties: Protein-like, brownish, amorphous substance. Does not pass through semipermeable membranes. Soluble in water at pH values of less than 1 or more than 6. Insoluble in organic solvents. Salted out of aqueous solutions at three-fourths saturation with $(NH_4)_2SO_4$. It is precipitated by alum, picric acid, and trichloroacetic acid. Positive Sakaguchi, biuret, ninhydrin, diazo, and Folin tests. Negative Fehling, Molisch, and xanthoproteic reactions. No decoloration of bromine and permanganate solutions. C = 55.39%; H = 6.66%; N = 9.93%. S, P, and halogens not detected. Acid hydrolysates

contain aspartic acid, glutamic acid, glycine, threonine, alanine, tyrosine, valine, leucine, phenylalanine, arginine, and three unidentified ninhydrin-positive spots. Electrophoresis reveals the presence of three components. Strong end-absorption in ultraviolet light, with a shoulder at 255 to 260 mμ. Infrared absorption curve given in reference 1.

Biological activity: Active against *D. pneumoniae*, *Shigella dysenteriae*, and *Sacch. sake* at the level of 10 μg per ml. Not active on other bacteria and fungi tested. Active against strain Nakayama of Japanese B encephalitis virus in mice.

Toxicity: LD_{50} (mice) 31 mg per kg intravenously, 55 mg per kg intraperitoneally, 161 mg per kg subcutaneously, and more than 1000 mg per kg orally.

Reference: 1. Matsumae, A. J. Antibiotics (Japan) **13A:** 143–154, 1960.

Cereviocidin

Produced by: Streptomyces sp., closely related to *S. cacaoi*.

Method of extraction: Absorbed on charcoal at pH 4.3 and eluted with 80 per cent aqueous acetone, pH 7.4. Eluate adjusted to pH 6.4 with HCl, concentrated, and extracted with ethyl acetate. The acetate is evaporated to dryness and the residue taken up in a small amount of chloroform. Filtered chloroform solution chromatographed on alumina, which is eluted first with chloroform and then with methanol, the latter removing another antibiotic present. Chloroform solutions combined with ligroin, chromatographed on alumina, and eluted with methanol. Methanol eluate combined with the chloroform solution that has been passed through the column and evaporated. The antibiotic separates and is recrystallized from hot ethyl alcohol.

Chemical and physical properties: Colorless needles; m.p. 249–250°C (decomposition). Soluble in methanol. Insoluble in ethanol, acetone, ethyl acetate, ether, chloroform, and benzene. Sparingly soluble in water. No characteristic ultraviolet absorption spectrum. pK = 4.5 (50 per cent methanol). $C_{22}H_{39}N_5O_4$. Calculated: C = 58.12%; H = 8.74%; N = 16.0%; Found: C = 57.76%; H = 8.79%; N = 16.01%. Positive Janovsky nitrogroup reaction; negative Tollen, biuret, Fehling, ninhydrin, Sakaguchi, maltol, and glucosamine tests.

Biological activity: Active on certain yeasts. Not active on representative bacteria, *M. tuberculosis* 607, or *C. albicans* in concentrations of 10 μg per ml.

Toxicity: Mice are killed by an intravenous injection of 150 mg.

Reference: 1. Yamashita, S. *et al.* J. Antibiotics (Japan) **8A:** 42–43, 1955.

Chartreusin

Produced by: Streptomyces chartreusis (1, 7), *Streptomyces* sp. (1, 8), *Streptomyces* sp. resembling *S. chartreusis* (9), *Streptomyces* sp. resembling *S. viridis* or *S. viridochromogenes* (3, 4, 6), and *Streptomyces viridochromogenes* (2, 5).

Synonyms: Antibiotic X 465A (9, 10); antibiotic 747 (3); antibiotic 6A36 (2); antibiotic G 72 (8); antibiotic 1293 (5).

Method of extraction: I. Mycelium extracted with 80 per cent acetone; broth-filtrate extracted with chloroform. Combined extracts concentrated to dryness *in vacuo*. Crystallized from acetone. Recrystallized from acetone-water. Sodium salt crystallized from water (1). II. Mycelium extracted with aqueous butanol; broth-filtrate extracted with butanol-butyl acetate (1:1). Extracted into water at pH 9.0 to 9.2, then backextracted into chloroform at pH 3.0 to 3.5. Concentrated extract purified by (a) removal of solvent and recrystallization from benzene-ligroin; or (b) removal of solvent, then chloroform solution of residue chromatographed on anhydrous calcium hydrophosphate and eluted with acetone-chloroform (1:3). Active fractions crystallized from aqueous acetone or acetone-ligroin (2). III. Broth-filtrate extracted with chloroform. Crude residue from acetone crystallized from acetone or aqueous acetone (6). IV. Broth and mycelium extracted with methylene chloride. Extract concentrated *in vacuo*, filtered, then reconcentrated. Addition of ethanol to boiling solution precipitates the antibiotic. Recrystallized from methylene chloride-ethanol (9).

Chemical and physical properties: Weakly acidic, glucosidic substance. *Anhydrous chartreusin:* Thin greenish yellow plates (3, 6, 9) or yellow prisms (2); m.p. 184–187°C (2, 3, 6, 9). Comparison of products from references 1, 3, 6, and 9 shows no depression of mixed melting points. Other characteristics are identical (9, 10). Soluble in chloroform, less so in acetone, slowly soluble in $NaHCO_3$ solution. More soluble in dilute acid than water. Soluble with inactivation in NaOH and Na_2CO_3 solutions (3, 6). Ultraviolet absorption spectrum maxima at 236, 266, 334, 380, 401, and 424 mμ (95 per cent ethanol) (6, 9). Slight differences in ultraviolet spectrum were noted (1, 2). Infrared spectrum given in references 1 and 2. $[\alpha]_D^{25} = +127.5° \pm 10°$ (c = 0.3 per cent in pyridine) or $-36.2° \pm 4°$

(c = 0.3 per cent in glacial HAc). Gives yellow-green color in alcoholic $FeCl_3$ (6) and dark green in $FeCl_3$ and dimethylformamide. *Chartreusin dihydrate:* Yellow rhombic plates, m.p. 234–235°C (crystallized from acetonitrile) (9); or greenish yellow crystals, m.p. 180°C. Fluoresces in ultraviolet light. Stable for several hours at pH 2 to 10 at 22°C (1). *Pentabenzoate:* m.p. 204–206°C (formed with benzoyl chloride in pyridine) (6). *Pentaacetyl derivative:* m.p. 270°C. Alkali-insoluble (6). Chartreusin decomposes both by heating to 250°C and by acid hydrolysis to give a weakly acidic aglycone, $C_{19}H_{10\text{-}12}O_6$. Acid hydrolysis products also include D-fucose and D-digitalose. *Aglycone:* Yellow needles, subliming at 260°C (crystallized from toluene or pyridine) or m.p. 310–311°C (6, 9). Soluble in aqueous alkali, but not in common organic solvents. Negative flavonol test (9). Chartreusin is believed to be a derivative of 2-phenylnaphthalene or 2,3-benzofluorene, containing a lactone group, two phenolic hydroxyls, and two oxygens of undetermined nature. There is an additional methyl or methylene group present, and one of the phenolic groups is glycosidically bound to a disaccharide chain composed of D-fucose and D-digitalose (10). C = 59.49, 59.69, 59.89, or 59.71%; H = 5.43, 5.30, 5.19, or 5.26% (1, 2, 6, 9). $C_{32}H_{34\text{-}36}O_{14}$ (6, 9, 10) or $C_{18}H_{18}O_8$ (1, 2, 4).

Biological activity: Active *in vitro* on gram-positive bacteria and mycobacteria. Active on certain gram-negative bacteria (*Neisseria* and *Hemophilus*). Active on *Nocardia asteroides*, various *Streptomyces* spp., and *Actinomyces bovis*. Active on various bacteriophages. Slightly active (10 to 40 μg per ml) on influenza A(FM-1), influenza B (Lee), HVJ (hemagglutinating virus of Japan or Sendai virus) in contact tests. Active in tissue culture on influenza PR 8. Not active on fungi or *Trichomonas* (1, 3, 7–9, 11). Not active *in vivo* on *Streptococcus hemolyticus*, pneumococcus type I, *Sal. schöttmuelleri*, *M. tuberculosis*, *C. albicans*, *Histoplasma capsulatum*, *Trypanosoma equiperdum*, *Endamoeba histolytica*, *Syphacia obvelata*, influenza A or SK viruses, or sarcoma 180 (9).

Toxicity: Chartreusin: LD_0 (mice) 2500 mg per kg subcutaneously (1). *Na chartreusin:* LD_{50} (mice) 250 to 300 mg per kg intravenously (1–3). Said to have a cumulative toxicity (1).

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10. Sternbach, L. H. *et al.* J. Am. Chem. Soc. **80:** 1639–1647, 1958.
11. Anzai, O. Virus **8:** 174–181, 1958.

Chloramphenicol

Produced by: Streptomyces venezuelae (5, 26). Also produced by *S. phaeochromogenes* var. *chloromyceticus* (6, 10), *S. omiyaensis* (10), *Streptomyces* sp. (22, 71), and by chemical synthesis (16).

Synonyms: Chloromycetin; sintomicetin; levomycetin; synthomycin; antibiotic 8-44.

Method of extraction: IA. Acidified broth-filtrate extracted with ethyl acetate, amyl acetate, cyclohexanone, butanol, or methyl isobutyl ketone. Extract solvent distilled off *in vacuo*. Residue extracted into diethyl ether or nitromethane. Chromatographed in aluminum oxide. Solvent in active fractions evaporated off; residue taken up in water and washed with petroleum ether. Concentration of aqueous solution gives crystals. Recrystallized from methylene dichloride, ethylene dichloride, and diethyl ether petroleum ether (1, 8, 17). IB. Broth-filtrate extracted with ethyl acetate at pH 8.5 to 9.0. Extracts concentrated, kerosene added, and the whole washed with 0.01 N H_2SO_4, 5 per cent $NaHCO_3$, and distilled water. Solvent dried, then distilled off *in vacuo* at 37°C. Cooling yields crystals (8). II. Broth-filtrate extracted with ether. Extract evaporated to dryness. Residue washed with hot benzene, leaving crystals. Recrystallized from benzene-methanol. Purified by chromatography on alumina from 30 per cent methanol in $CHCl_3$. Eluate concentrated to dryness. Residue crystallized from chloroform-5 per cent methanol (3). III. Adsorbed from broth-filtrate at pH 5.6 on activated carbon. Eluted with acidic acetone. Eluate adjusted to pH 4.0 to 5.0 and solvent distilled off *in vacuo*. Taken up in ether. Ether dried, concentrated, decolorized, and evaporated to dryness. Residue taken up in hot water. Antibiotic crystallizes out on drying (17, 22).

Chemical and physical properties: Neutral substance (1). *Free base:* Colorless needles or elon-

gated plates; m.p. 149.7–150.7°C (corrected) (1) or 144–147°C (3). Sublimes without decomposition in high vacuum (15). Very soluble in methanol, ethanol, butanol, propylene glycol (150.8 mg per ml), acetone, ether, and amyl acetate (1, 3). Soluble in cyclohexanone, methyl isobutyl ketone, nitrobenzene, nitromethane, and ether. Very sparingly soluble in water (2.5 mg per ml at 25°C), acid, alkali, hot benzene, and chloroform (3). Insoluble in petroleum ether, cold benzene, cold 5 per cent sodium bicarbonate, and vegetable oils (3, 8, 17, 18). Ultraviolet absorption spectrum maximum at 278 mμ ($E^{1\%}_{1cm}$ 298) (water or 0.1 *N* HCl) and 279.0 to 279.5 mμ (0.1 *N* NaOH) (3). $[\alpha]_D^{25} = -25.5°$ (ethyl acetate) (1) or +18° (ethanol) (17). Positive $KMnO_4$ and ferrous hydroxide tests (for oxidized nitrogen). Biuret test is not characteristically positive; green crystals of a copper salt deposit on standing (17). Negative Sakaguchi, Pauly, $FeCl_3$, Molisch, Benedict (boiling) (3, 8), thiosemicarbazone, boiling or cold alcoholic silver nitrate, and periodic acid tests (17). Stable at room temperature in aqueous solution at pH 2 to 9 for >24 hours; stable to boiling for 5 hours (1). Crystallographic data given in reference 40. C = 41.11%; H = 3.89%; N = 8.60%; Cl (nonionic) = 21.71% (1). $C_{11}H_{12}O_5N_2Cl_2$. Molecular weight 323.1. Structural formula given in Chapter 6. Acid or alkaline hydrolysis products include *dichloroacetic acid* and a basic substance, $C_9H_{12}N_2O_4$, having only $^1/_{100}$ the activity of chloramphenicol against *Shigella paradysenteriae* (17). *Acetyl derivative of chloramphenicol:* Crystalline; m.p. 141–142°C (17). A variety of derivatives and analogues of chloramphenicol have been reported (21, 25, 29, 31, 36, 41, 57). Many of these are biologically active, but none is of greater interest than the parent compound. Certain generalizations concerning the relationship of biological activity to structure in this group have been made (42, 59).

Biological activity: In vitro: Active on gram-positive and gram-negative bacteria, rickettsiae, and psittacosis group (1, 3, 4, 19). Not active on filamentous fungi or yeasts (4, 19). Active on *Actinomyces israelii* and *Nocardia farcinica*, but not other pathogenic nocardias (23). Active on certain pleuropneumonia-like organisms (38). Amebistatic to *Endamoeba histolytica* at 125 to 250 μg per ml, depending on conditions of the test (28). Active on *Tetrahymena pyriformis* (54). At bacteriostatic concentrations, inhibits intracellular growth of coliphage T-1 in *E. coli* (50). Not active on gonococci phagocytized by HeLa cells (62), but kills *Brucella* phagocytized by guinea pig leucocytes (71). Inhibits phagocytosis of *Staph. aureus* by human polymorphonuclear neutrophils at 15 μg per ml (45). Prevents nitrate assimilation by the fungus *Scopulariopsis brevicaulis* grown in static surface culture, but does not prevent assimilation of other N-containing compounds or nitrate when the culture is grown under submerged conditions (70). Chloramphenicol inhibits synthesis of protein and nucleic acids (67). d-Chloramphenicol is biologically inactive, but does not interfere with the activity of chloramphenicol (30). Chloramphenicol decomposition products produced by the action of certain bacteria have either a growth-stimulating effect on bacteria, or interfere with the growth-inhibitory action of the antibiotic (39). *In vivo:* Active (in mice) on *K. pneumoniae* (type A), *Shigella paradysenteriae*, *D. pneumoniae* (type I), *Streptococcus hemolyticus*, *S. viridans*, *Borrelia novyi*, *Pasteurella multocida*, *P. pestis*, *Sal. gallinarum*, *Malleomyces pseudomallei*, *Clostridium tetani*, and *C. septicum*. (4, 12, 14, 27, 47, 66). Slight activity on *Treponema pallidum* (rabbits) (19). Active at high doses on *Endamoeba histolytica* in rats; suppressive but no curative effect in dogs (28). Active on *Plasmodium berghei* (mice), *P. gallinaceum* (chicks), and *P. cathemerium* (canary) (32). Interferes with the killer action of *Paramecium aurelia* (37). Antitoxin activity in mice against *Sal. bareilly* endotoxins (58). Active on *Rickettsia prowazekii* (chick embryo), *R. orientalis* (chick embryo and mice), *R. akari*, *R. mooseri*, *R. rickettsii*, *R. tsutsugamushi*, *R. burneti*, *R. conorii*, and the rickettsia that causes North Queensland tick typhus (1, 2, 13, 33). Active on psittacosis, lymphogranuloma venereum, and mouse pneumonitis virus (2, 9, 52). Inactive on St. Louis and Japanese encephalitis viruses, variola, influenza A (PR 8), A-1, and B, fixed rabies, distemper, Newcastle disease, vaccinia, polio (Lansing and Y-SK), Theiler's intestinal, mumps, chick bronchitis, and laryngotracheitis viruses (2, 4, 19). Active on stone-fruit virus in artificially inoculated cucumbers, and on tobacco mosaic virus in tomato seedlings when the chloramphenicol is introduced by vacuum (51). Inhibits the virus tumor of *Rumex acetosa* L (11). Active on crown-gall tissue of tomato (53). Protects wheat seedlings from *Xanthomonas translucens* infections when they are grown in a solution containing the antibiotic (60). Active on sarcoma 180 in mice when tumor cell suspension is mixed with the antibiotic before inoculation and a pellet of the drug is implanted subcutaneously 1 day after inoculation with such tumor cells (64). Prolongs motility and life-span of human spermatozoa

(63). When administered to the silkworm (*Bombyx mori* L), growth increases but silk production decreases (46). When fed to weanling pigs, weight and feed efficiency increase (48). Prevents contamination in grain fermentation (43). Preserves beef (44) and fish (20).

Toxicity: LD_{50} (mice) 150 mg per kg (1), 195.4 mg per kg (18), or 245 mg per kg (4) intravenously. LD_{50} (mice) 1320 mg per kg intraperitoneally, and 2640 mg per kg orally (18). LD_{50} (rats) 175.5 mg per kg intravenously (in 60 per cent propylene glycol), or 279.4 mg per kg, same route (in 50 per cent acetamide). LD_{50} (rabbits) 117.0 mg per kg intravenously (in 100 per cent propylene glycol) (18). LD_{50} (10-day-old chick embryo, allantoic route) 4.8 mg per egg (68). Repeated parenteral administration results in anemia in dogs (4), but this was later reported to be indirectly a result of malnutrition secondary to the anorexia produced by the drug (56). Anemia, unrelated to malnutrition, was reported in ducks receiving chloramphenicol (55). Blood dyscrasias occur rarely in certain human beings (35, 69), but no such reactions were noted in 2142 patients who received massive doses of B complex and ascorbic acid concurrently with the antibiotic (61). *Plants and plant cells:* Transiently toxic to *Allium cepa* root cells at 1000 ppm (34). Causes chlorosis and reduction of dry weight in wheat seedlings grown in 25 μg per ml (60). Nontoxic at 125 μg per ml to normal tomato plant tissue, but inhibits crown-gall tissue of the same plant at this level (53). *Animal and tumor cells:* Highest concentration permitting epithelial cell migration in tissue culture is 4.0 mg per ml (72). Least injurious doses to spleen of chick embryo and human skin cells in tissue culture are 165 to 300 and 135 to 275 μg per ml, respectively (49). Minimal dose causing degeneration of HeLa cells is 500 μg per ml (65).

Utilization: Active on a variety of diseases caused by gram-positive and gram-negative bacteria, spirochetes, rickettsiae, and certain viruses (69).

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Chlortetracycline

Produced by: Streptomyces aureofaciens (15) and *S. sayamaensis* (105).

Synonyms: Aureomycin, biomycin, duomycin, flamycin, syntomycin, aureomykoin. (See also tetracyclines.)

Method of extraction: IA. Whole broth adjusted to pH 1.4, ammonium oxalate and Arquad 16 (50 per cent solution in isopropanol) added, pH adjusted to 8.5, and the whole extracted with methyl isobutyl ketone. Water added to extract, and pH adjusted to 0.5. Crystals form on prolonged stirring. Purified by treatment with sodium hydrosulfide at pH 1.8 for 10 minutes, then at pH 0.5 for 20 hours (137). Crystallized from hot water on cooling and addition of HCl. Conversion from hydrochloride to base: Substance slurried in dimethylformamide. Addition of $NaHCO_3$ gives precipitate. Toluene solution of precipitate azeotropically distilled. Cooling gives precipitate. Recrystallized from benzene (88). IB. Whole broth adjusted to pH 2.9 and filtered. Arquad C (a commercial mixture of alkyl and alkenyltrimethylammonium chlorides, principally dodecyltrimethylammonium chloride) and calcium chloride are added to filtrate, and pH adjusted to 9.0 to precipitate a chlortetracycline-organic base-

bivalent metallic ion complex. Precipitate slurried in water and a methanolic solution of the precipitate adjusted to pH 2.5 to precipitate impurities. Solution adjusted to pH 7.0 to precipitate the antibiotic (131). II. Adsorbed from broth-filtrate on Florisil or charcoal columns. Developed with acidic alcohol or acetone at pH <5.0 under ultraviolet light. First band (blue) discarded; yellow band which follows is active. Eluate concentrated *in vacuo*. Concentrate taken up in butanol, reconcentrated, and precipitated from concentrate with absolute ether (15). III. Earth metals present in, or added to the whole broth at pH 7.0 to 8.5 precipitate chlortetracycline as a salt. All solids filtered off, and wet cake extracted with n-butanol, isopropanol, or sec-butanol at pH 1.2 to 1.4 (adjusted with sulfuric or hydrochloric acids). Extracts containing the sulfate shaken with sodium chloride solution to salt out chlortetracycline. (This has the effect of increasing the distribution coefficient of chlortetracycline where K = C solvent/C aqueous.) Solvent concentrated under reduced pressure to incipient precipitation, chilled, and pH adjusted to 0.8 to precipitate the antibiotic. Extracts containing the hydrochloride are concentrated under reduced pressure at 45–55° C, added to β-ethoxyethanol-ethanol-HCl, and cooled (60–62, 121). Three purification procedures are used: (a) Crude chlortetracycline dissolved in such basic compounds as triethylamine, ammonia, ethanolamine, or morpholine in a lower alcohol (such as ethanol) or a lower alkoxy-lower alkanol (2-ethoxyethanol or ethylene chlorohydrin), filtering off insoluble impurities and acidifying (85). (b) Aqueous solution of crude chlortetracycline treated at pH 3.0 with an anionic sulfuric acid derivative such as di-(2-ethylhexyl) sulfosuccinate ("Aerosol OT"). The salts thus formed are extracted into methyl isobutyl ketone, ethylene dichloride, or n-propyl acetate, decolorized, then concentrated under reduced pressure at <55°C and acidified (86). (c) Heavy metal chelating agents are used to sequester impurities before precipitating the antibiotic as a salt. Such agents include various aminopolycarboxylic acids, such as (ethylenedinitrilo)tetraacetic acid and others (132).

Chemical and physical properties: Amphoteric (88). *Free base:* Yellow, acicular to bladed crystals (15, 88); m.p. 168–169°C (decomposition) (12). Soluble in water to 0.5 to 0.6 mg per ml at 25°C. Very soluble in aqueous solutions above pH 8.5, dioxane, pyridine, Cellosolves, and carbitol (12, 15). Soluble in methanol, ethanol, butanol, acetone, ethyl acetate, and benzene. Insoluble in ether and petroleum ether. Ultraviolet absorption spectrum maxima at 230, 262.5, and 367.5 mμ (0.1 *N* HCl); at 255, 285, and 345 mμ (0.1 *N* NaOH) (12) and at 230, 275, and 367.5 mμ (water) (35). Infrared spectrum given in reference 15. $[\alpha]_D^{23} = -275.0°$ (methanol) (12). Treatment with alcoholic $FeCl_3$ gives a green-brown color with reflected light and a reddish color with transmitted light (12). Fluoresces intensely yellow in neutral or slightly alkaline solution, changing to blue in marked alkaline solution or after heating. No fluorescence in acid solution (22). Alkaline fusion products include 5-chlorosalicylic acid, dimethylamine, and ammonia. Other degradation products given in reference 40. Reductive dehalogenation with 10 per cent palladium on charcoal and 1 mole of triethylamine yields tetracycline (68). Acid degradation products include biologically active anhydrochlortetracycline (water is removed from ring C) (104). Heating produces biologically inactive, nontoxic isochlortetracycline (127, 128). Chlortetracycline is 7-chloro-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,-10,12,12a, pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide. $C_{22}H_{23}N_2O_8Cl$: C = 55.10%; H = 4.90%; N = 5.72%; Cl = 7.27%. Structural formula (39, 88) given in Chapter 6. Forms complexes with metal halides (41), and salts with metals and acids. *Hydrochloride:* Clear vitreous lemon-yellow tabular or orthorhombic crystals (12, 15). Decomposes without melting at >210°C (12, 20), or darkens at 208°C; m.p. 234–236°C (decomposition) (88, 121). Soluble in water to 14 mg per ml at 25°C (12). Soluble in methanol. Slightly soluble in ethanol. Soluble in acetone to 0.13 mg per ml at 25°C. Ultraviolet absorption spectrum maxima at 229, 251, 265, and 370 mμ (pH 4.3, 0.1 *M* KH_2PO_4 buffer); at 223, 240, 248, and 276 mμ (pH 8.9, 0.1 *M* K_2HPO_4 buffer) (15) and at 224 mμ (ϵ = 29,480) 254 mμ (ϵ = 14,635), 287 mμ (ϵ = 14,378) and 346 mμ (ϵ = 7,008) (in 0.25 *N* NaOH) (106). $[\alpha]_D^{25} = -235°$ (c = 1 per cent in water) (88). Crystallographic data given in references 12, 15, and 16. $pK_a' = 3.4$, 7.4, and 9.2 (39). Most stable at about pH 2.0. Stable at pH 1 to 10 at low temperatures for 18 hours, but loses 50 per cent of activity at 37°C overnight (3). In distilled water, 65 per cent of initial activity remains after heating at 100°C for 15 minutes. In pH 7.0 phosphate buffer, 0.062 *M*, under the same conditions, only 0.44 per cent of the activity remains (107). *Methanolate:* Yellow plates; m.p. 172–174°C (decomposition). Very stable to drying (88). *Ca salt:* Amorphous. Decomposes over a range of 200–300°C with gradual darkening at lower temperature (121).

Biological activity: Bacteriostatic. Active on

gram-positive and gram-negative bacteria, certain protozoa, rickettsiae, and the psittacosis group. Not active on fungi. *In vitro:* Active on streptococci (0.3 to 1.25 μg per ml), diplococci (0.1 to 0.3 μg per ml), staphylococci (<0.6 μg per ml), *E. coli*, *Aerobacter aerogenes* (<5.0 μg per ml), *K. pneumoniae* (1.0 to 5.0 μg per ml), *Hemophilus influenzae* (2.0 μg per ml), *Brucella suis* and *B. abortus* (<0.75 μg per ml), and *Actinomyces israelii*. Not active at 20 μg per ml on *Pseudomonas* or *Proteus* (11). Active on pleuropneumonia-like organisms (PPLO) (0.23 to 1.25 μg per ml) (54). Inhibits bacteria-free *Tetrahymena* and two colorless flagellates at >0.1 μg per ml. At 0.85 μg per ml early growth is stimulated (30). Promotes growth of protozoa living on *Polytomella* at 10 to 50 μg per ml (31), and at a narrow range of concentration (about 0.1 μg per ml) in a bacteria-free culture. At higher concentrations, the protozoa are inhibited (30). Interferes with the killer action of *Paramecium aurelia* (53). Active on *Endamoeba histolytica* and *Trichomonas vaginalis* (14). Stimulates growth of *C. albicans* at >0.1 mg per ml (65). Inhibits CO_2 fixation in the dark by *Scenedesmus obliquus*, but increases sucrose accumulation and stimulates photosynthesis up to six times the normal rate at 1.5×10^{-4} *M* and above (64). Inactive on free coliphage T3, but prevents adsorption and growth of the phage if the host cell is preincubated with subbacteriostatic concentrations of the antibiotic. Infected cells are more susceptible to the antibiotic than normal cells (66). Activity adversely affected in presence of serum (2). Growth inhibition of *E. coli* is reversed by glycine, inosine, lumichrome, and riboflavin; in the case of the latter, competitively (67). Resistance develops relatively slowly (2). Anhydrochlortetracycline, although having only a fraction of the antibacterial activity of chlortetracycline, is much more active than the parent compound on actinomycetes (104). *In vivo:* Active in various animals on *Streptococcus pyogenes*, *S. hemolyticus*, *Staph. aureus*, *D. pneumoniae*, *B. anthracis*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Listeria monocytogenes*, *K. pneumoniae*, *Sal. typhosa*, *E. coli*, *Pr. vulgaris*, and *Ps. pseudomallei* (2, 4, 5, 11, 93, 135). Cell-free preparations of *C. albicans*, prepared by sonic vibration, are lethal to mice when given in combination with chlortetracycline, but innocuous alone (130). Active on *Endamoeba histolytica* infections in rats, dogs, and rabbits (37, 118), *Plasmodium cathemerium* in the canary, and *P. berghei* in mice (38). Some activity on *Toxoplasma gondii* infections in mice (77). When administered in the diet, it increases the susceptibility of *Aedes aegypti* to *P. gallinaceum* infections, but decreases it in *Anopheles* (58). Active in rats on experimental polyarthritis (PPLO strain L_4) (13), and in chick embryos on a PPLO strain causing arthritis in the goat (120). Active in eggs, mice, and guinea pigs on rickettsiae of Rocky Mountain spotted fever, murine, scrub, North Queensland tick and epidemic typhus, North African tick bite fever, Boutonneuse fever, Q fever, and rickettsial pox (8, 28, 103). Active in mice on infections caused by the following viruses: feline pneumonitis (but not *in vitro*) (18), a gray lung fever (21), mouse hepatitis (32), vaccinia (also active *in vitro*) (33), Rous sarcoma (*in vitro*), tested in chickens (47), herpes simplex (*in vitro*) (111) and the lymphogranuloma-psittacosis group (8). Not active in mice on influenza B, canine distemper, rabies street virus, Newcastle disease virus, Venezuelan equine encephalomyelitis, poliomyelitis (MEF-1 strain) (8, 52), myxoma virus (chick embryo), fibroma, myxomatosis viruses (rabbits) (78), or Bittner's milk virus (78). Moderate activity on cat ascarids; no activity on hookworms or tapeworms (79). Anthelmintic activity in mice (57) and horses (83). Protects against hemorrhagic shock in dogs (54). May stimulate Walker carcinosarcoma (rat) (87). Enhances growth of the following transplanted tumors: rat carcinoma 175-G, Crocker rat carcinoma, mouse sarcoma 180, and mouse adenocarcinoma EO771 at 1.6 to 3.2 mg per 100 gm of body weight when given for 4 to 10 days. At 8 mg per 100 gm for 18 days, completely inhibits some 1- to 3-day transplants of Crocker rat carcinoma, but not 10-day transplants (27). No effect on mammary tumors (C3H mice) or Lucké kidney adenocarcinoma (frogs) (78). In plants, chlortetracycline controls, by seed treatment, black rot of rutabaga (*Xanthomonas campestris*) (92). Some control of tomato crown-gall (*Agrobacterium tumefaciens*) (23). Active on bacteria-free gall tissue (112). Stimulates growth of radish plants (72) and lupine root (*Lupinus*) (87). Increased growth rate has been reported in the following when chlortetracycline was added to the diet: mice (81); protein-deficient (98) or vitamin-deficient (99) rats; chicks (16); pullets (70); goslings (46); geese (100); bobwhite quail (122); turkey poults (19); lambs (95); weanling (93) and disease-free pigs (97); calves (69, 114); yearlings (69); steers (126); horses (89); cats (91); dogs (48); undernourished children (74); and young men (90). Alkali-degraded chlortetracycline has no growth-promoting effects in the chick (26) Chlortetracycline is degraded to biologically inactive isochlortetracycline in the rat intestinal tract (133). Dietary chlortetracycline: (a) *In-*

creases: feed efficiency (46, 48, 95, 96, 114, 124); egg hatchability (70); lamb carcass grades (95); utilization of calcium and phosphorus (chick) (42); nitrogen retention (cockerels) (101), (calves) (124); intestinal permeability to organic nitrogen (rats) (75); serum carotenoid levels in chicks (45); vitamin A deposition in vitamin A-deficient rats (113); nicotinic acid levels in the liver (rats) (115); intestinal synthesis of vitamins (rats) (108); blood reducing sugar levels (calves) (102), (pigs) (80). (b) *Causes:* toxic symptoms and death in guinea pigs at levels causing growth stimulation in other animals; believed caused by an increase in the numbers of *Listeria monocytogenes* in the gut, and subsequent disease symptoms (116). (c) *Reduces:* intestinal weight in pigs (117) and chicks (123); urinary nitrogen excretion in cockerels (101) and dairy calves (125). (d) *Has a sparing effect on:* vitamin B_{12} (chick) (16), (pigs) (73); nicotinic acid and folic acid (chick) (25); thiamine (chick) (44); pantothenic acid (pigs) (73); all vitamins (rats) (108); tryptophan (chicks) (24); choline (rats) (this activity was destroyed on heating) (49); protein (rats) (98); manganese (chicks) (43).

Toxicity: LD_{50} (mice) 134 mg per kg (1) or 50 to 100 mg per kg (5, 107) intravenously, 3 to 4 gm per kg subcutaneously (5). Mice tolerate 1500 mg per kg orally, 200 mg per kg intraperitoneally (1). LD_{50} (rats) 118 mg per kg intravenously (1), 13.55 gm per kg orally (119). LD_{50} (chick embryo) 12.0 mg (134). Guinea pigs tolerate 300 mg per kg subcutaneously, but are killed by 200 mg per kg intraperitoneally (1). Very toxic to guinea pigs orally (116). Dogs are killed by a dose of 150 mg per kg intravenously (5). Nonirritating at 1 per cent to rabbit eyes (11). *Fish and amphibians:* Toxic to guppies at 1 mg per ml in 10 to 30 minutes (59). At dilution of 1:10,000 to 1:25,000, inhibits tadpole metamorphosis (76). *Insects:* Toxic to the granary weevil (*Sitophilus granarius*) and the confused flour beetle (*Tribolium confusum*) at 0.3 to 0.5 gm per 14 gm of grain. At these levels the lesser grain borer (*Rhizopertha dominica*) is stimulated reproductively (63). Kills the adults of the rice weevil (*Calandra oryzae*) and the small rice weevil (*C. sasakii*) after exposure to 0.2 gm in a jar for 2 days (84). *Plants:* Toxic to the growth of lentil and pea seedlings at dilutions of 5×10^{-5}, and has a temporary blocking action on the production of chlorophyll. Not toxic to rape (*Brassica napus*) at 1×10^{-3} (55). Na-K chlorophyllin prevents characteristic chlorotic toxicity symptoms in beans and cucumbers normally produced when the plants are sprayed with certain concentrations of chlortetracycline. *Cells:* Least injurious doses for spleen cells (from chick embryo) and human skin cells in tissue culture are 150 to 300 μg per ml and 100 to 200 μg per ml, respectively (94). Cytotoxic to *Allium cepa* root cells at 50 ppm (29). Nontoxic to normal plant cells at 50 μg per ml, although crown-gall cells are inhibited at this level (112). Minimal dose causing inhibition of HeLa cells is 125 μg per ml (129).

Utilization: Active against a variety of infections caused by gram-positive and gram-negative bacteria, actinomycetes, rickettsiae, and psittacosis group (6, 7, 9–11, 14, 17). It is widely used in veterinary medicine. Used in feeds to augment growth rate of poultry, calves, pigs, etc. Used in preservation of foods: fish (109), poultry (82), meat (110), milk (50), and spinach (133). Prevents contamination in grain fermentation (71).

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Chromin

Produced by: Streptomyces sp. resembling *S. antibioticus* (1).

Method of extraction: Broth-filtrate extracted with butanol or amyl alcohol at pH 6 to 7. Taken up in methanol, and fractionally precipitated with ether (2, 3).

Chemical and physical properties: Tetraene. Fine white needles. Colors at 145–150°C, but does not melt up to 220°C. Crude chromin soluble in methanol, water-saturated butanol, chloroform, and alkaline water. Crystalline chromin is not soluble in most organic solvents or water. Soluble in NaOH and acetic acid. Ultraviolet absorption spectrum maxima at 281, 292.5, 305, and 320 mμ. Infrared spectrum given in reference 3. Positive Fehling test (on heating). Negative ninhydrin, biuret, Molisch, Millon, $FeCl_3$, and Sakaguchi tests. Questionably positive Tollen test. Most stable at neutrality. C = 58.19%; H = 7.81%; N = 2.29%. No S or halogen (2, 3). Some information on hydrolysis products given in reference 3.

Biological activity: Active on fungi and yeasts at 0.16 to 2.5 μg per ml. Not active on bacteria. Activity reduced by human serum (2, 3).

Toxicity: LD_{50} (mice) 36 mg per kg intraperitoneally (3).

References:

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3. Wakaki, S. *et al.* J. Antibiotics (Japan) **6B:** 247–250, 1953.
4. Katagiri, K. *et al.* Shionogi Kenkyusho Nempô **7:** 715–723, 1957.

Chromomycins

Produced by: Streptomyces griseus.

Method of extraction: Separated and purified by chromatography and fractional precipitation.

Chemical and physical properties: Complex containing five components, A_1 to A_5. Previously reported substances B and C were found to be formed by transformation of A under various conditions and with various agents. *A_3:* Bright yellow powder; m.p. 183°C (decomposition). Reputed to have ultraviolet and infrared absorption spectra differing from other antibiotics. $[\alpha]_D^{20} = -26°$ (c = 1 per cent in ethanol).

Biological activity: A_3: Active on *Staph. aureus* and *B. subtilis* at 0.10 and 0.05 μg per ml (2), respectively and on other gram-positive bacteria. Slightly active on mycobacteria. Active on vaccinia virus; slightly active on influenza virus (1). Active in rats or mice on Yoshida sarcoma, Ehrlich carcinoma, hepatoma AH 130, hepatoma MH 134, sarcoma 180 (all ascites type), and leukemia SN 36. Not active on nitromin-resistant AH 7974 (2).

Toxicity: A_3: LD_{50} (mice) 2.12 mg per kg intraperitoneally. Rats, hamsters, rabbits, cats, and dogs die on administration of 0.25 to 1.0 mg per kg, but tolerate 0.1 to 0.2 mg per kg. Mice tolerate 400 mg per kg orally (2). Toxic to chicken embryo fibroblasts *in vitro* (1).

Utilization: Favorable effect in some human beings with neoplastic disease (3).

References:

1. Aramaki, Y. *et al.* Repts. Takeda Research Lab. **14:** 60–91, 1955.
2. Tatsuoka, S. *et al.* Proc. Japan. Cancer Assoc., 17th Meeting 23–24, 1958.
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Chrysomycin

Produced by: Streptomyces sp.

Synonym: Authors (1) state that the ultraviolet spectrum shows similarities to aureolic acid.

Method of extraction: Extraction from filtrate with organic solvents at acid or alkaline reaction. Dried mycelium treated with Skellysolve C to remove inactive pigments, then exhaustively extracted with boiling ethyl acetate. Green-brown

extract passed through Florisil columns to remove most dark-colored impurities. While being shielded from bright daylight to prevent further pigment formation, the columns are washed with the same solvent to remove the active material. Bright yellow eluate concentrated *in vacuo*, and recrystallized by dissolving in hot pyridine and adding ethanol or by dissolving in hot acetic acid and adding water.

Chemical and physical properties: Slender greenish yellow needles or rods; m.p. 255–260°C (decomposition). Can be sublimed at 240°C without loss of activity. Neutral substance, practically insoluble in water and petroleum ether; slightly soluble in methanol, higher alcohols, and ethyl acetate; and more soluble in pyridine, glacial acetic acid, and dioxane. Soluble in concentrated HCl and can be recovered unchanged from it by adding water. Dry crystals are photosensitive, and turn brown on exposure to light. Stable from pH 3 to 7 and to heating to 100°C. $[\alpha]_D^{22} = +16°$ (c = 1 per cent in acetic acid). Ultraviolet maxima at 247 to 287 mμ and a broad band between 390 to 400 mμ. Gives inactive red solution in alkali. On hydrogenation with platinum oxide as catalyst and glacial acetic acid as solvent, 4 moles of hydrogen are taken up. The colorless product, recrystallized from hot ethanol, is devoid of biological activity and melts at 208°C; ultraviolet maxima at 240 and 355 mμ. Analysis of this hydrogenation product gave a formula of $C_{22}H_{28}O_7$. Molecular weight 360. C = 65.46%; H = 6.96%; O = 27.47%. From this analysis the tentative formula $C_{22}H_{20}O_7$ is proposed for chrysomycin, since the original compound could not be analyzed satisfactorily.

Biological activity: Active against *B. cereus* phage at 0.01 μg per ml, and other phages, including staphylophage, streptophage, enterococci phage, and cholera phage at 0.2 mg per ml by the paper-disc method. Phagocidal to five bacterial viruses. Has antibacterial activity (active mainly against gram-positive bacteria) and slight antifungal activity.

Toxicity: Mice (20 gm) tolerate 2 mg in peanut oil intraperitoneally; 5 mg by the same route produces transient paralysis and loss of appetite.

Reference: 1. Strelitz, F. *et al.* J. Bacteriol. **69:** 280–283, 1955.

Cinnamycin

Produced by: Streptomyces cinnamomeus (1) f. *cinnamomeus* (4).

Method of extraction: Broth-filtrate passed through a column of IRC-50 (H^+) and eluted with 0.1 *N* HCl. Neutralized to pH 6.0 with IR-4B. Effluent concentrated *in vacuo* and freeze dried. Solid dissolved in 80 per cent methanol (aqueous) and chromatographed on alumina. Development with aqueous methanol (1). Countercurrent distribution (n-butyl alcohol and a volatile ammonium acetate buffer, 0.2 *M*, pH 5.4) indicates the presence of two substances, one of which is unrelated to cinnamycin (2).

Chemical and physical properties: Cream-colored powder. Basic polypeptide. Soluble in water, hydrated alcohols, and glacial acetic acid. Insoluble in ether (1). Ultraviolet absorption spectrum shows end-absorption at 230 mμ. Infrared spectrum given in reference 2. Aqueous solutions are levorotatory. Stable at pH 2 to 9 for 30 minutes at 92°C. Photo-stable. Not inactivated by pepsin or trypsin (1). Dialyzes through cellophane. Isoelectric point pH 5.0 (2). Contains N and S; no halogen. Positive Sakaguchi and biuret tests. Negative ninhydrin, $FeCl_3$, Molisch, maltol, Tollen, and reducing sugar tests. No sulfhydryl or disulfide groups (1). Acidic and basic hydrolysis products include aspartic acid, arginine, glutamic acid, proline, phenylalanine, valine, mesolanthionine, and β-methyl lanthionine ($C_7H_{14}N_2O_2S$), which has also been isolated from subtilin and nisin (1, 2).

Biological activity: Active on gram-positive rods and mycobacteria (5 to 55 μg per ml); very active on *Clostridium botulinum* (0.085 μg per ml); not active on gram-positive cocci, gram-negative bacteria, or yeasts (1). Tested against phytopathogens and compared with duramycin (4).

Toxicity: LD_{50} (mice) 5 to 10 mg per kg intraperitoneally; 400 mg per kg or less is nontoxic subcutaneously (3).

References:

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3. Ambrose, A. M. Antibiotics & Chemotherapy **4:** 1242–1244, 1954.
4. Lindenfelser, L. A. *et al.* Antibiotics & Chemotherapy **9:** 690–695, 1959.

Cladomycin

Produced by: Streptomyces lilacinus (2).

Method of extraction: Broth extracted with ethyl acetate. Extract concentrated *in vacuo* at <40°C. Precipitated from concentrate on addition of petroleum ether. Taken up in ethyl acetate, washed with HCl (pH 2.0) and NaOH (pH 10.0) solutions, concentrated *in vacuo* at <40°C, and precipitated as before. Purified by chromatography on a cellu-

lose column. Eluted with methanol (2, 3). Can also be extracted from broth with ether or chloroform (1).

Chemical and physical properties: Dark red substance. Soluble in alcohols, acetic esters, acetone, benzene, and chloroform. Insoluble in petroleum ether and water. No specific ultraviolet absorption spectrum. Contains no halogen (1–3).

Biological activity: Active at 0.0039 to 3.0 μg per ml against gram-positive and gram-negative bacteria, including mycobacteria, *Pseudomonas*, and *Proteus*. Not active on fungi (1, 2).

Toxicity: LD_{50} (mice) 724.5 mg per kg intravenously (1, 2).

References:

1. Nakazawa, K. *et al.* J. Antibiotics (Japan) **9B:** 81, 1956.
2. Nakazawa, K. *et al.* Ann. Repts. Takeda Research Lab. **16:** 111–115, 1957.
3. Japanese Patent 3241, April 30, 1959.

Coelicolorin

Produced by: Streptomyces coelicolor.

Method of extraction: Extraction of solid cultures with acetone at acid pH. Precipitation of active substance from acetone solution with water; extraction with benzene. Further purification by chromatography on alumina.

Chemical and physical properties: Indicator: red up to pH 5, violet at pH 6 to 7, and blue higher than pH 8. Soluble in water at alkaline reaction. Very soluble in acetone, ethyl acetate, and chloroform. Soluble in alcohol, methanol, benzene, and ether. Insoluble in petroleum ether. m.p. 142–146°C.

Biological activity: Active mainly against gram-positive bacteria.

Toxicity: LD_{50} (mice) about 500 mg per kg intraperitoneally.

Reference: 1. Hatsuta, Y. J. Antibiotics (Japan) **2:** 276–277, 1949.

Coerulomycin

Produced by: Streptomyces coerulescens.

Synonym: Similar to chartreusin.

Method of extraction: I. Precipitation from culture-filtrate at pH 2.5 to 3.0. Precipitate extracted with acetone; extract concentrated. A brown precipitate forms. Precipitate extracted with alcohol. Crystallization from alcohol yields crude crystals contaminated with pigments; the pigments are removed by chromatography of alcoholic solution on aluminum oxide. The yellow acetonic eluate is concentrated and the antibiotic crystallized from the alcohol. II. Extraction of the culture medium with butanol, chloroform, or ethyl acetate at neutrality, followed by further purification as in previous method.

Chemical and physical properties: Crystals in form of platelets. Darken and decompose at 181–183°C. Molecular weight (Rast) 660. Moderately soluble in chloroform, less soluble in acetone and alcohol, and poorly soluble in water. At an alkaline reaction, 20 mg will dissolve in 1 ml of water. No N or S. Light-absorption maxima at 240, 270, 340, 380, 400, and 430 mμ.

Biological activity: Active against gram-positive bacteria and phages (especially actinophages). Active against influenza virus.

Toxicity: In mice, 200 mg per kg are tolerated subcutaneously, and 300 mg per kg orally.

Reference: 1. Brajnikova, M. G. *et al.* Antibiotiki **2(6):** 16–20, 1957.

Collinomycin

Produced by: Streptomyces collinus. This organism also produces rubromycin (1).

Method of extraction: See rubromycin.

Chemical and physical properties: Amphoteric substance. Orange prisms; m.p. 280–282°C. Moderately soluble in chloroform, acetone, and dioxane; slightly soluble in ether and lower alcohols; practically insoluble in petroleum ether, water, and sodium bicarbonate. *Na salt:* Violet substance. Red-violet in 2 *N* NaOH. Ultraviolet absorption maxima at 535 and 575 mμ. Carmine-red in concentrated H_2SO_4, with maxima at 480 and 525 mμ. A pyridine-methanol solution treated with titanium trichloride turns olive-green, then red. A yellow-red dioxane solution becomes pale yellow on treatment with sodium hyposulfite. This color reaction is reversed by standing in the air. Contains no nitrogen. C = 59.41%; H = 4.01%; O = 34.83%; O—CH_3 = 16.2%. *Acetate:* Yellow needles; m.p. 228–230°C. *Benzoate:* Lemon-yellow needles; m.p. 226°C. Prolonged heating in H_2SO_4-containing acetone yields a decomposition product: orange needles, m.p. 273–275°C, with ultraviolet absorption maxima at 515 and 529 mμ (ether). Gives a red-violet color in $NaHCO_3$ (2).

Biological activity: Active on *Staph. aureus* (2).

References:

1. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
2. Brockmann, H. and Renneberg, K. H. Naturwissenschaften **40:** 166–167, 1953.

Cryptocidin

Produced by: Streptomyces sp.

Method of extraction: Mycelium extracted with

methanol. Extract concentrated *in vacuo* to precipitation. Precipitate dried, washed with absolute ethanol, and taken up in 90 per cent acetone. Solution concentrated, filtered if necessary, and reconcentrated to precipitate the antibiotic.

Chemical and physical properties: Hexaene. Crystalline; m.p. 100–115°C (with foaming). Very soluble in 0.01 *N* NaOH and 80 per cent acetone; soluble in methanol; scarcely soluble in water, ethanol, and acetone. Ultraviolet absorption spectrum shows major maxima at 341 ($E^{1\%}_{1cm}$ 380), 358 ($E^{1\%}_{1cm}$ 585), and 380 mμ ($E^{1\%}_{1cm}$ 605), and minor peaks at 290, 305, and 320 mμ. Negative biuret, Sakaguchi, Molisch, Fehling, ninhydrin, and $FeCl_3$ tests. Dark blue in H_2SO_4. Yellow in acidic solution, slowly becoming brown at alkaline pH. Loses 40 per cent of activity in 0.01 *N* NaOH at 100°C for 5 minutes. Stable at the same pH for 5 hours at 5°C. Paper chromatographic behavior given in reference 1.

Biological activity: Active on *Staph. aureus* at 6.8 μg per ml, *B. subtilis* at 12.5 μg per ml, *Candida utilis* at 1.2 μg per ml, and *P. chrysogenum* at 6.0 μg per ml.

Toxicity: LD_{50} (mice) 135 mg per kg orally, >10 mg per kg intravenously.

Reference: 1. Sakamoto, J. M. J. J. Antibiotics (Japan) **12A:** 21–23, 1959.

Crystallomycin

Produced by: Streptomyces violaceoniger var. *cristallomicini* (1).

Synonym: Similar to amphomycin. Crystallomycin differs from amphomycin in that (a) it is more active against experimental pneumococcal infections, and (b) it has more antibacterial activity in liquid media. Complete cross-resistance was not observed between these two substances (1).

Toxicity: LD_{50} (mice) 124 mg per kg intravenously, 109 mg per kg intraperitoneally, 220 mg per kg subcutaneously, and over 1500 mg per kg orally (2). Pharmacological data given in reference 2.

References:

1. Shorin, V. A. and Shapovalova, S. P. Antibiotiki **4(1):** 77–81, 1959.
2. Goldberg, L. E. Antibiotiki **4(4):** 63–66, 1959.

Cyanomycin

Produced by: Streptomyces cyanoflavus.

Method of extraction: Extracted from broth-filtrate at neutral or alkaline pH with chloroform, methylene chloride, or tetrachloroethane. Back-extracted into acidic water. Mycelium extracted two or three times with 0.1 *N* HCl, then back-extracted into chloroform at pH 8.5. Process repeated several times. Final acidic water extract neutralized and cooled to precipitate cyanomycin. Recrystallized from hot water.

Chemical and physical properties: Monoacidic base. Possibly contains a quinone group. Dark blue needles; m.p. 128°C (decomposition). Soluble in water to 0.5 mg per ml; in hot water to 5 mg per ml. Soluble in lower alcohols, acetone, chloroform, and methylene chloride. Slightly soluble in carbon tetrachloride, ethyl acetate, benzene, and ether. Insoluble in petroleum ether and cyclohexane. Decomposes in 0.1 *N* NaOH, but not in 0.1 *N* HCl. pK = 4.98. Ultraviolet absorption spectrum maxima at 240 ($E^{1\%}_{1cm}$ 550), 278 ($E^{1\%}_{1cm}$ 2140), and 384 mμ ($E^{1\%}_{1cm}$ 780) in 0.1 N HCl; at 239 ($E^{1\%}_{1cm}$ 760), 278 ($E^{1\%}_{1cm}$ 1180), 310 ($E^{1\%}_{1cm}$ 730), and 384 mμ ($E^{1\%}_{1cm}$ 500) in phosphate buffer at pH 5.0; at 238 ($E^{1\%}_{1cm}$ 1010), 310 ($E^{1\%}_{1cm}$ 1470), and 378 mμ ($E^{1\%}_{1cm}$ 320) in carbonate buffer at pH 9.0; at 239, 293, and 370 mμ in 0.1 *N* NaOH. Infrared spectrum given in reference 1. Negative Fehling, biuret, ninhydrin, and $FeCl_3$ tests. Red solution in H_2SO_4 changes to green-yellow with zinc powder; effect reversible by H_2O_2 but not by bubbling air through the solution. Aqueous solution changed to light blue with zinc powder; change unaffected by atmospheric O_2; and becomes colorless on addition of H_2O_2. Weakly alkaline solution reversibly changed to light blue-green with H_2O_2. Aqueous solution becomes yellow-brown with H_2O_2; and blue, then colorless with zinc powder. Aqueous solution becomes yellow-brown on addition of $NaHSO_4$, then red with H_2O_2. $C_{15}H_{12}N_2O_2$: C = 69.22%; H = 5.16%; N = 10.76%. *Picrate:* Purple-red needles; m.p. 157.5°C.

Biological activity: Active on *E. coli* at 4 μg per ml and *Staph. aureus* at 6 μg per ml. Much less active (10 to 60 μg per ml) on other bacteria tested. Not active on fungi or yeasts.

Toxicity: Mice tolerate 25 mg per kg intravenously.

Reference: 1. Funaki, M. *et al.* J. Antibiotics (Japan) **11A:** 143–149, 1958.

Cycloheximide

Produced by: Streptomyces griseus (1, 26, 77) (also produces streptomycin or streptovitacin), *Streptomyces* sp. related to *S. virido-* or *olivochromogenes* (52), *S. noursei* (53) (also produces nystatin), *Streptomyces* sp. differing from *S. griseus* (71) (also produces a stereoisomer of cycloheximide, "naramycin B"), *Streptomyces* sp. (41), and *S. albulus* (culture produces (72) two forms of cyclo-

heximide, nystatin, and antitumor antibiotic E 73).

Synonyms: Actidione (1), naramycin A (71), antibiotic A 67 (41).

Remarks: Substances having greatly inferior or no antifungal activity, *inactone* (50, 51) and *nonactin* (52), have been isolated from cycloheximide-containing broths. Streptovitacin, an antitumor substance, has also been isolated from certain cycloheximide broths (77). A cycloheximide-like substance (m.p. 105–106°C) that was one third as active a cycloheximide was reported (31). It may be similar to naramycin B (see below).

Method of extraction: I. Adsorbed on carbon or carbon-Celite at pH 2.0 or other acid pH from broth-filtrate. Eluted with anhydrous or 80 per cent acetone, or anhydrous methanol. Eluate distilled off, residual solution extracted with chloroform. Extract decolorized with carbon and evaporated. Purified by countercurrent distribution (benzene-water). Crystallized from amyl acetate (1, 2), or from anhydrous ethyl ether on pouring into petroleum ether. Further purified by chromatography on Darco G-60-Celite, with 20 per cent aqueous acetone as solvent and 60 to 100 per cent acetone as developer. Crystallized from active fractions on adding amyl acetate and seeding. Recrystallized from hot water or 30 per cent methanol (2). II. Broth-filtrate extracted with amyl acetate. Mycelium extracted with acetone. Extracts concentrated, then extracted with amyl acetate. Amyl acetate-extracts combined and concentrated *in vacuo*. Nonactin precipitates on standing. Mother liquor chromatographed on aluminum oxide and developed with chloroform-methanol (19:1). Further purified by countercurrent distribution (CCl_4-$CHCl_3$-MeOH-water, 55:55:75:25). Crystallized from ether by seeding; recrystallized from acetone-petroleum ether (52). III. Adsorbed on carbon from broth-filtrates at pH 5.5 to 6.0. Eluted with acetone at pH 4.0 to 4.5 followed by water. Eluates adjusted to pH 6 to 7, and acetone distilled off *in vacuo*. Aqueous residue extracted into chloroform. Chloroform neutralized and an oil which separates, removed. Chloroform distilled *in vacuo*. Residue extracted with water, clarified, and acetone added to 20 per cent. Chromatography on Darco G-60-Celite 545, developed with 20 per cent acetone, and eluted with aqueous acetone containing successively less water. Active fractions are distilled *in vacuo* to remove the acetone, extracted into chloroform, and chloroform distilled off. Residue crystallized from amyl acetate. Recrystallized from 30 per cent methanol (53).

Chemical and physical properties: Neutral substance. Rectangular or square colorless plates; m.p. 115–117°C (1, 2, 53). Very soluble in all common organic solvents except saturated hydrocarbons. Soluble to 2.1 gm per 100 ml of water and 7 gm per 100 ml of amyl acetate (2). Ultraviolet absorption spectrum maximum at 287 mμ (ϵ = 36.7) (4). Infrared data given in reference 4. $[\alpha]_D^{25}$ = −2.8° (c = 9.6 per cent in methanol) (1), or +6.8° (c = 2 per cent in water) (2). pK_a' = 11.2 (4). Positive Fearon-Mitchell test (primary or secondary alcoholic hydroxyl) (4). Crystals stable for several hours at 100°C. Aqueous solutions stable for several hours at 60 but not 70°C (26). C = 64.16%; H = 8.17%; N = 5.13%. $C_{15}H_{23}NO_4$. Structural formula (2, 4) is given in Chapter 6. β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide. Inactivated by alkali at room temperature (1), giving a fragrant volatile product, d-2,4-dimethylcyclohexanone, as well as propionaldehyde-2,2-diacetic acid (4). *Cycloheximide diacetate:* m.p. 148–149°C (2). $[\alpha]_D^{25}$ = +24.6° (c = 3.7 per cent in methanol). Biologically inactive (1). *Monoxime:* m.p. 203–204°C (2, 4). *Semicarbazone:* m.p. 182–183°C (2, 4).

Biological activity: In vitro: Active on a variety of plant pathogens at 0.125 to 20 μg per ml, including members of the phycomycetes, ascomycetes, basidiomycetes, and Fungi Imperfecti (8). Most active on yeasts of the *Saccharomyces* genus; less so on *Torula* or *Hansensia*. Not active on *Kloeckera apiculata*. Active on most alcoholic fermentation processes; slightly active on respiration; not active on lactic fermentation (31). Not active on gram-positive or gram-negative bacteria (15). Active on *Tetrahymena geleii*, *Euglena gracilis* var. *bacillaris* (colorless), and *Endamoeba histolytica* (25). Inhibits the growth of certain members of *Chlorophyceae*, *Xanthophyceae*, and *Bacillariophyceae* at 50 ppm or less, but has no effect on *Myxophyceae* (68). Active *in vitro* on influenza A (PR 8) virus by direct inactivation of the virus particles (67). Partially inhibitory levels of cycloheximide in a medium on which *Allomyces arbuscula* is grown give rise to the formation of male and female gametangia on portions of the sporophyte (22). Inhibits protein and nucleic acid synthesis in *Sacch. carlsbergensis*. At minimal growth-inhibitory levels, synthesis of desoxyribonucleic acid and protein is completely inhibited, but ribonucleic acid synthesis continues slowly for some time (66). *In animals*: Temporary clearance of *E. histolytica* from macaques (13), rats, and dogs (58). Inhibits growth of the following in mice, rats, or hamsters: Miyono adenocarcinoma, Mecca

lymphosarcoma (69), and Eagles KB epidermoid carcinoma (76). Moderately inhibits Bashford carcinoma 63, adenocarcinoma E 0771, carcinoma 1025, Wagner and Ridgeway osteogenic sarcomas, Harding-Passey melanoma, Flexner-Jobling carcinoma (69), and RC mammary carcinoma (70). Slight inhibition of Crocker sarcoma 180 in mice (32). Inhibition (at 1 mg per day) of weight increase in mice inoculated with Ehrlich ascites carcinoma, an effect which ceases with withdrawal of the drug (40). *In plants:* Cycloheximide controls: *Powdery mildew* of: beans (*Erysiphe polygoni*) (3); dewberry (*Sphaerotheca humuli*) (20); onion (17); squash (*Erysiphe cichoracearum*) (19); rose (*Sphaerotheca pannosa* var. *rosae*) (5); grape (*Uncimula necator*) (49); and cherry (*Podosphaero oxyacanthae*) (46). *Leaf spot* of cherry (9) and artificial infections with *Coccomyces hiemalis* of cherry trees (34). *Smut* (covered) of oats (*Ustilago kolleri*) (21) and wheat (*Tilletia* sp. (30) and *T. foetida* (75)). *Rust of:* wheat (*Puccinia graminis* var. *tritici*) (43); mint (*Puccinia menthae*) (17); turf (stem rust, *Puccinia graminis*) (48); safflower (*P. carthami*) (54); red cedar (*Gymnosporangium juniperi-virginianae*) (45); white pine (63); apple leaf (cedar rust) (42); and asparagus (semicarbazone used) (74). *Turf diseases: Sclerotinia homeocarpa* and *Helminthosporium* sp. infections (18, 28); "fading out" (*Curvularia lunata*) (35); leaf spot phase of "melting out" (*Helminthosporium vagans*) (59). *Miscellaneous: Sphacelotheca sorghi* infections of sorghum (38); *Rhizoctonia* and *Verticillium* infections of potato stems (36); brown rot of peach (*Sclerotinia fructicola*) (49); rot of Delicious apples (73); oak wilt (*Endoconidiophora fagacearum*) (44); wood-rotting fungi, including *Poria microspora* (16); cucumber scab (*Cladosporium cucumerinum*) (10); black leg of cabbage (*Phoma lingam*) (60); derrite disease of coffee (*Phyllosticta coffeicola*) (61); *Dactylium dendroides* mildew infection of mushrooms (56); post-harvest decay (*Botrytis* and *Rhizopus*) of strawberries (64). *Other activity:* At very low concentrations, increases seed germination of oats and Madrid sweet clover, sprout growth of oats, and top growth of both oats and clover. Added to soil, increases bacterial and actinomycete population and nodules on sweet clover, but decreases the fungal population (33). Holds down mold count on harvested black raspberries (27).

Toxicity: LD_{50} (mice) 150 to 160 mg per kg intravenously (1) and subcutaneously (31); 375 mg per kg orally (31). LD_{50} (guinea pigs) 60 mg per kg subcutaneously; (rabbits) 17 mg per kg intravenously; (cats) 4 mg per kg intraperitoneally; (rats) 2.7 mg per kg subcutaneously, 5 mg per kg orally, and 2.5 mg per kg intravenously (12, 31). Intravenous injection of 1 mg per kg in dogs is followed by vomiting within a few minutes (7). Cycloheximide was found to be very repellent to rats (12). *Plants:* Inhibits germination of the following seeds by soaking for 4 hours (ppm): radish (100), pea (>100), wheat (100). Soaking for 30 minutes at 100 ppm has no effect on seed germination of cantaloupe, spinach, or cucumber seeds (6). Toxic to tomato and bean plants at 1 ppm, wheat at 10 ppm, geranium and peach at 100 ppm; not toxic to strawberry at 1000 ppm (applied as sprays) (14, 30). Stunts alfalfa seedlings at 0.4 μg per ml in a soil solution (29). pH of the spray was found to be a factor in toxicity of cycloheximide (37). Nontoxic when applied to cantaloupe plants as a 4-ppm spray (47). Use of chlorophyll in cycloheximide sprays reduces the toxicity of the antibiotic on beans without affecting antifungal activity (65). Injurious to roses at concentrations of 0.1 to 0.4 ppm when sprayed on the young leaves (42). Produces cytological aberrations, including prophase inhibition, in *Pisum sativum* (39) and *Allium cepa* (11) root cells, as well as in HeLa cells at 12.5 μg per ml (57). *Human beings:* Nausea observed following cycloheximide injections in human beings (7). Toxic by intrathecal route, but not intravenously (23). Crystalline cycloheximide and its concentrated solutions are highly irritating to the skin, producing a reddening and sloughing (62).

Utilization: Plant disease. Used commercially for control of turf diseases and cherry leaf spot (62). Usefulness of cycloheximide is reduced by the fact that at effective therapeutic levels it is often toxic to the host plant (55). Sweet wine preservation (31). Therapeutic effect in one case of cryptococcic meningo-encephalitis, and gave a remission in one case of coccidioidal meningitis (24).

STEREOISOMERS OF CYCLOHEXIMIDE: Cycloheximide has four centers of asymmetry permitting isomers.

Naramycin B

Produced by: Streptomyces sp. differing from *S. griseus*. This culture also produced cycloheximide (71).

Chemical and physical properties: Colorless plates; m.p. 109–110°C. Ultraviolet absorption spectrum maximum at 292.5 mμ (log ε, 1.49) and a shoulder at 232 mμ (methanol). Infrared data given in reference 71. $[\alpha]_D^{12.5} = +50.2°$ (c = 2 per

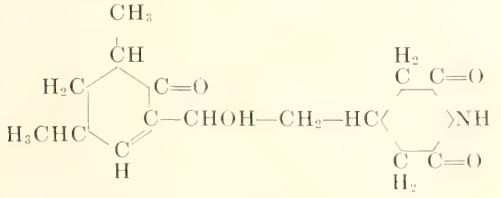
cent in methanol). $C_{15}H_{23}O_4N$: C = 64.3%; H = 7.80%; N = 4.90%. More heat-stable than cycloheximide. *Acetate:* m.p. 150.5–152°C. $[\alpha]_D^{12.5}$ = +62.5° (c = 2 per cent in methanol). *Benzoate:* m.p. 159–160.5°C. $[\alpha]_D^{12.5}$ = +54.6° (c = 1 per cent in methanol) (71).

Biological activity: Has 32 per cent of the activity of cycloheximide on *Saccharomyces sake* (71).

Inactone

(See remarks under cycloheximide.)

Chemical and physical properties: Colorless flat needles; m.p. 116°C. Ultraviolet absorption spectrum maximum at 330 mμ ($E_{1cm}^{1\%}$ 50) (ethanol). Infrared spectrum similar to that of cycloheximide (51). $[\alpha]_D^{25}$ = −55° (c = 2 per cent in water). Inactone is one of eight possible dehydrocycloheximides having the following structure (50, 51):



Biological activity: None.

Nonactin (52)

Chemical and physical properties: Neutral substance. Colorless needles; m.p. 147–148°C. Optically inactive. Ultraviolet absorption spectrum maximum at 264 mμ (weak). Infrared spectrum given in reference 52. Chemically unreactive substance. $C_{30}H_{48}O_9$.

Biological activity: Very little or none.

Isocycloheximide

Method of extraction: Mother liquors of cycloheximide crystallization batches evaporated under reduced pressure. Extraction with chloroform of aqueous concentrate. Chloroform-extract decolorized with carbon and concentrated to syrup. Isopropanol added, and after storage for 3 months at 5 to 10°C, crude isocycloheximide collected. Further purification by chromatography on carbon, followed by recrystallization.

Chemical and physical properties: Same infrared light absorption spectrum as cycloheximide but slight differences in the low cm^{-1} regions; m.p. 95–102°C. $[\alpha]_D^{25}$ = +32° (c = 1.0 per cent in CH_3·OH).

Biological activity: Thirty per cent of the cycloheximide activity against *Saccharomyces pastorianus.*

Toxicity: Thirty per cent of the intravenous toxicity of cycloheximide in mice.

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Cycloserine

Produced by: Streptomyces orchidaceus (5, 9, 35), *S. garyphalus* strains (22), *S. lavendulae* strains (2, 6, 21), *S. roseochromogenes* (1, 20), and *S. nagasakiensis* (28).

Synonyms: Oxamycin (3), orientomycin (orientmycin), antibiotic K 300, special substance 2 (20),

antibiotic 106-7 (22), antibiotic PA 94, seromycin, and antibiotic JN 21 (35).

Method of extraction: I. Adsorbed from culture-filtrates on Dowex 2 (OH^- form) or other strong basic anion exchange resin and eluted with dilute sulfuric acid. Eluate decolorized with charcoal at pH 7.0. Cycloserine precipitated as the silver salt on addition of silver nitrate at pH 6.5. Converted to the base by decomposing an aqueous solution of the salt with dilute HCl, and freeze drying. Crystallized from water with alcohol or acetone (9, 35, 36). II. Broth clarified with charcoal, adjusted to pH 1.5 to 3.0, and adsorbed on IR-120 (OH^- cycle). Elution with 0.1 to 0.2 *N* NH_4OH. (a) Chromatographed on Amberlite XE-98 (OH^+ form) and eluted with 0.3 *N* acetic acid. Eluate treated with charcoal, adjusted to pH 10.0 to 10.5, concentrated, and treated with isopropanol, ethanol, or acetone to separate out impurities. Adjustment of supernatant to pH 6.0 with acetic acid and cooling precipitated cycloserine. Crystals dissolved in water and adjusted to pH 12 with KOH. Solution treated with ethanol and isopropanol and mixture filtered. Filtrate cooled, adjusted to pH 5.8 with glacial acetic acid to reprecipitate cycloserine (8, 23). (b) Chromatographed on alumina with 50 per cent methanol as solvent and developer. Concentration first precipitates impurities, then cycloserine. Recrystallized from hot methanol or from water on addition of ethanol (2).

Chemical and physical properties: Amphoteric substance (6, 20). Fine white needles; m.p. 153–156°C (decomposition) (5, 6, 8, 9), or 149–150°C (29). Sublimes *in vacuo* at 100°C (35). Very soluble in water; soluble in methanol; slightly soluble in acetone; very slightly soluble or insoluble in most other organic solvents (2). Ultraviolet absorption spectrum maximum at 226 mμ ($E^{1\%}_{1cm}$ 402) (water, pH 6.0) (8). Infrared spectrum given in references 1, 9, and 20. $[\alpha]_D^{25} = +112°$ (c = 5 per cent in 2 *N* NaOH). $[\alpha]_{5461}^{25} = 137° \pm 2°$ (c = 5 per cent in 2 *N* NaOH). pK_a = 4.4 to 4.5 and 7.3 to 7.4 (8, 9, 20). Exists in solution as a dipolar ion (9). Positive $FeCl_3$ test (2). Green color with cupric ions in aqueous solution; reddish brown with iron; biological activity destroyed in both cases (35). Decomposes in glacial acetic acid (22). Rf = 0.4 (80 per cent ethanol-water), producing a brown-yellow color with ninhydrin. Highly diffusible (3). Stable to heat (2). Stable to alkali; unstable at acid or neutral pH (5). Crystallographic data on the anhydrous and hydrous forms given in reference 22. C = 35.4%; H = 5.98%; N = 26.9%. Equivalent weight, 101 to 104. Structural formula, D-4-amino-3-isoxazolidinone ($C_3H_6N_2O_2$) (8, 9) is given in Chapter 6. Prolonged acid hydrolysis yields DL-serine and hydroxylamine. In solution, cycloserine dimerizes to 2,5-bis(aminoxymethyl)-3,6-diketopiperazine; m.p. 190–200°C (decomposition) (8). *Calcium salt:* m.p. 215–221°C (decomposition) (8, 22). *Magnesium salt:* m.p. 224–228°C (22). *Silver salt:* Square platelets (35). Cycloserine and some of its analogues have been synthesized (10, 30). *L-4-Amino-3-isoxazolidinone:* m.p. 148–149°C. $[\alpha]_D^{20} = -119°$ (c = 1 per cent in water) (29). *D-1-Cycloserine* (racemic): m.p. 136–137°C (29).

Biological activity: In vitro: Very limited (6.25 to 500 μg per ml) activity on gram-positive and gram-negative bacteria. Most active on mycobacteria at pH 6.4 to 7.0. Activity otherwise unaffected in a range of 4.5 to 8.0. Not active on fungi (4, 11, 14, 15). Active on bacteria-free *Endamoeba histolytica*; $^1/_{100}$th as active on *E. histolytica* in bacteria-containing cultures (25). Competitively inhibits the growth factor for mycobacteria, mycobactin; cycloserine contains an iso-oxazole ring, mycobactin an oxazole ring (7). Reversibly and noncompetitively inhibits catalase activity in mycobacteria, as well as purified beef liver catalase. Also blocks purified peroxidase and the peroxidase activity of tubercle bacilli (39). Blocking the amino group destroys the activity on *E. coli* (32). *In vivo:* More active *in vivo* than could be expected from *in vitro* tests. Active in mice on infections caused by *Borrelia novyi*, *Staph. aureus*, *K. pneumoniae*, *Sal. schottmuelleri*, *E. coli*, *Ps. aeruginosa*, *Streptococcus pyogenes*, and *D. pneumoniae* (4). Active in mice, but not *in vitro*, on *Nocardia asteroides* (37). Moderately active on mouse leprosy (33). Active against tuberculosis in monkeys, but has limited to no activity in mice, guinea pigs, and rabbits because of differences in attainable blood levels of the drug (16). Slightly active in mice and eggs on *Rickettsia mooseri*, and in eggs on feline pneumonitis. Not active on SK encephalomyelitis or swine influenza virus. Not active *in vivo* on protozoa, except slightly on *Plasmodium gallinaceum* (chickens) and *Endamoeba histolytica* (rats). No anthelmintic activity (4).

d- Versus l- and Racemic Cycloserine

d-Cycloserine is most active on bacteria; l-cycloserine is almost inactive. The racemic mixture has activity equal to the d-form, although it contains only 50 per cent of the d-form (26). l-Cycloserine is twice as active as the racemic mixture, and 10 times as active as d-cycloserine against human strains of *M. tuberculosis* (27). The racemate is more active than either the d- or l-form against pneumococcus type I, but not against *Sal. enteritidis* (30). The racemic form is more active

than the d-form against *E. coli* in a synthetic medium. The l-form is inactive unless employed in an asparagine-containing medium, in which it is as active as the d-form (27). d-Cycloserine is more active against tuberculosis in mice than the dl- or l-forms (29).

Toxicity: LD_{50} (mice) 1.81 to 2.5 gm per kg intravenously, 2.87 to 4.3 gm per kg, intraperitoneally, 2.8 to >5 gm per kg subcutaneously, and 5.29 ± 0.20 gm per kg orally. LD_{50} (rat) >5.0 gm per kg subcutaneously and orally (18, 19). Rats and mice show slight depression at 0.25 gm per kg orally; transient coma at 1 gm per kg, and deep coma at 4 gm per kg. Four gm per kg induce convulsive seizures in rabbits (17). Four hundred mg per kg per day are severely neurotoxic to monkeys (31). Side reactions in human patients include lethargy, convulsions, and disorientation (13). Pyridoxine administered simultaneously is said to reduce the toxicity of cycloserine (40).

Utilization: Used in tuberculosis as a last resort in combination with other drugs (12, 34). Used for certain other bacterial infections (11, 24) and leprosy (38).

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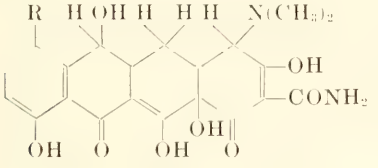
Demethyltetracyclines

Produced by: Streptomyces aureofaciens (mutant of the original Duggar chlortetracycline-producer). I is produced in chloride-free media; II in chloride-containing media (1).

Synonyms of 7-chloro-6-demethlytetracycline: Demethylchlortetracycline, declomycin, ledermycin.

Chemical and physical properties: I. 6-Demethyltetracycline hydrochloride hemihydrate: m.p. 203–209°C (decomposition). $C_{21}H_{24}N_2ClO_{8.5}$: C = 52.52%; H = 5.34%; N = 6.05%; Cl = 7.51%; H_2O = 1.96%. $[\alpha]_D^{25} = -259°$ (c = 0.5 per cent in 0.1 *N* H_2SO_4). Ultraviolet absorption spectrum maxima essentially the same as the corresponding 6-methylated tetracycline (1). II. 7-Chloro-6-demethyltetracycline sesquihydrate: m.p. 174–178°C (decomposition). $C_{21}H_{24}N_2ClO_{9.5}$: C = 51.13%; H = 4.93%; N = 6.00%; Cl = 7.39%; H_2O = 4.45%. $[\alpha]_D^{25} = -258°$ (c = 0.5 per cent in 0.1 *N* H_2SO_4). Both I and II and their epimers (synthetic) are considerably more resistant to alkaline or acidic degradation than other tetracyclines. Ultraviolet absorption spectrum maxima of II are essentially the same as corresponding 6-methylated tetracyclines (1). Structural formulas (1):



I: R = H
II: R = Cl

Biological activity: I and II: 24 and 75 per cent, respectively, of the activity of chlortetracycline against *Staph. aureus*. II is also active against *Staphylococcus*, *Streptococcus*, *Klebsiella*, and pneumococcal infections in mice (2).

Toxicity: II has a low order of toxicity. Absorbed slowly from the gastrointestinal tract. Produces very high serum concentrations, probably because it is cleared slowly by the kidney (1).

Utilization: Effective in the treatment of a number of human diseases (3).

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Desertomycin

Produced by: Streptomyces flavofungini. This strain also produces flavofungin.

Method of extraction: Extracted with organic solvents from broth and mycelium.

Chemical and physical properties: Snow-white, glittering hexagonal crystals; m.p. 189–190°C. Low solubility in water, absolute alcohols, ether, and acetone; higher solubility in aqueous alcohols. Positive bromine, $KMnO_4$, ninhydrin, and Kuhn-Roth (C-methyl) tests. Very soluble in neutral aqueous solution. $C_{33}H_{60-62}O_{14}N$. Does not contain N—Me or acetyl groups. Gives hydrogenated and acetylated products.

Biological activity: Active on gram-positive and gram-negative bacteria (1 to 25 μg per ml). Inhibits leukemic and Ehrlich ascites carcinoma cells *in vitro*. Cystostatic (10 μg per ml) and cytolytic (50 to 100 μg per ml) on fibroblast, HeLa, and Crocker sarcoma cells by tissue culture method.

Toxicity: LD_{50} (mice) 1.35 mg per kg intravenously, 2.6 mg per kg intraperitoneally, 5.3 mg per kg subcutaneously, and 12 to 15 mg per kg orally.

Reference: 1. Uri, J. *et al.* Nature, London **182:** 401, 1958.

Diazomycins

Produced by: Streptomyces ambofaciens.

Synonyms: Related to azaserine, DON, and alazopeptin.

Method of extraction: Concentration of the filtered broth to 5 to 10 per cent of original volume; 10 volumes of methanol added and the filtrate concentrated. The aqueous concentrate passed through a Dowex 1 acetate column. The effluent contains 6-diazo-5-oxo-l-norleucine (DON). The column is eluted with 1 per cent phosphate buffer at pH 7.0. Two active fractions are eluted. Fraction A chromatographed on Celite or silica gel and eluted with the system phosphate buffer-n-butanol-isopropanol. The active component, diazomycin A, converted to its lithium salt and crystallized. Fraction B, which still contains some diazomycin A, is purified by countercurrent distribution between phenol and water. Two peaks are observed. Both fractions chromatographed on Dowex 1 acetate resin. One of the fractions is homogenous: diazomycin C. The other fraction can be split into two components, diazomycin A and a new component, diazomycin B.

Chemical and physical properties: Aliphatic diazo compounds. Very labile at acidic pH values. Most stable between pH 6.0 and 8.0. Light absorption spectrum maxima at 275 and 245 mμ. Extinction coefficients at 275 mμ: diazomycin A, 520; B, 550; C, 340; at 245 mμ: A, 315; B, 340; C, 210. Ninhydrin reaction: A and C, light gray-blue; B, intense blue. Solubility in methanol: A and C, readily soluble; B, slightly soluble. Rf values (80 per cent isopropanol): A, 0.6 to 0.7; B, 0.2 to 0.3;

C, 0.8 to 1.0. Infrared absorption spectra given in reference 1. Elementary analysis: A, C = 44.34%; H = 5.08%; N = 17.22%. B, C = 43.15%; H = 5.59%; N = 19.22%. C, C = 46.03%; H = 5.93%; N = 23.82%.

Biological activity: Active against certain yeasts and bacteria. *B. subtilis* used as the assay organism. Diazomycin B is the most active biologically, then A, then C. Active in animals against sarcoma 180, adenocarcinoma 755, and to a lesser extent against leukemia 1210.

Reference: 1. Rao, K. V. *et al.* Antibiotics Ann. 943–949, 1959–1960.

Dihydrostreptomycin

Produced by: Catalytic hydrogenation of streptomycin (1), *Streptomyces humidus* (23) (organism also produces humidin), and *Streptomyces* sp. (32).

Synonym: Antibiotic 23572 (23).

Method of extraction: I. See references 1, 14, and 23 for chemical details on hydrogenation. II. Extracted from culture-filtrate with n-butanol (or isoamyl alcohol) containing 3 per cent lauric acid, at pH 7.5. Organic layer shaken with aqueous acid at pH 2.0. Aqueous extract shaken with ether (23). III. Crystalline sulfate is precipitated from an aqueous solution (pH 5.5) on addition of glycerol (or formamide). Heating to 50–60°C, addition of methanol to turbidity, temperature maintained at 60°C, stirring (21).

Chemical and physical properties: In dihydrostreptomycin, the aldehyde of streptomycin is reduced to the corresponding carbinol (see formula in Chapter 6). *Trihydrochloride:* White powder or fine needles containing methanol (lost on heating); m.p. 185–190°C (decomposition). Powder soluble in methanol to 1 gm per ml; needles to 45 mg per ml (1, 11). No characteristic absorption in ultraviolet light. Infrared spectrum given in reference 23. $[\alpha]_D^{25} = -89.5°$ (c = 0.98 per cent in water) (1). Crystallographic data given in references 11 and 14. Differs from streptomycin in these reactions: (a) not inactivated by hydroxylamine or cysteine; (b) not degraded to maltol in alkali; (c) more stable in alkali; and (d) forms many complex mixed acid salts but not the double $CaCl_2$ salt that streptomycin forms (1–3, 15). Positive Sakaguchi test. Negative Fehling (boiling), phenol-H_2SO_4, and silver mirror tests. Slightly positive Tollen test. $C_{21}H_{41}N_7O_{12}\cdot 3HCl$: C = 36.5%; H = 6.21%; N = 3.91%. Equivalent weight, 690. $pK_a' = 7.7$. Yields streptidine and dihydrostreptobiosamine on alkaline hydrolysis (1, 3, 23). *Sulfate:* Trapezoidal plates or small nonhygroscopic platelets. *Anhydrous salt:* m.p. 250°C or 255–265°C (decomposition). Soluble in water, and moderately soluble in aqueous methanol. $[\alpha]_D^{25} = -88°$ (c = 1 per cent in water) (10, 11, 23). Crystallographic data given in reference 11. *Trihelianthate:* Plates (23); m.p. 215–225°C (decomposition) (1), 222–225°C (23), or 224–330°C (decomposition) (3). *Reineckate:* Needles. Sinter at 194°C; m.p. 204–206°C (decomposition) (3). Forms metal chelates: Dihydrostreptomycin-copper: Dark blue substance. Decomposes at 178–183°C (19).

Biological activity: In vitro and *in vivo* activity is of the same order as streptomycin (2, 9) except that against *Salmonella* it is one third to one fourth as active *in vitro*, and *in vivo* it is even less active (6). Dihydrostreptomycin has some rickettsiostatic activity, but less than streptomycin (5). *In vivo:* A dihydrostreptomycin-dependent strain derived from a pathogenic strain of *Sal. typhosa* was nonpathogenic for mice, but capable of stimulating immunity (16). Inhibits the killer action of *Paramecium aurelia* (13). Active on crown-gall (*Agrobacterium tumefaciens*) of geranium (17).

Toxicity: Sulfate: LD_{50} (mice) 262.5 mg per kg intravenously (4), 1700 mg per kg intraperitoneally (23), 1600 ± 108 mg per kg (6), 910 mg per kg (30), or 1200 mg per kg (22), or 1800 mg per kg (23) subcutaneously. LD_{50} (1-week-old chicks) 743 mg per kg intramuscularly; (4-week-old chicks) 1868 mg per kg, same route (20). LD_{50} (10-day-old chick embryos, allantoic cavity) 80.6 mg (29). As in the case of streptomycin, the reduced toxicity of the pantothenate salt of dihydrostreptomycin was reported. *Pantothenate:* LD_{50} (mice) 1550 mg per kg (expressed as base) subcutaneously (22). This was confirmed by some (24, 26); others disagreed (25). Dihydrostreptomycin was reported to have less severe vestibular toxicity than that of streptomycin in cats (7) and in human beings (8, 27). However, it produces a delayed deafness which is more serious than that produced by streptomycin (12, 31). Nephrotoxic (8). *Plants:* Produces apochlorosis and growth inhibition in barley (18).

Utilization: Clinically effective in tuberculosis and in numerous other infectious diseases due to gram+ and gram− bacteria (27, 28).

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Dihydrodesoxystreptomycin

Produced by: This antibiotic is a chemical derivative of streptomycin.

Chemical and physical properties: More stable in solution than dihydrostreptomycin. Structural formula given in Chapter 6.

Biological activity: Equally as active as dihydrostreptomycin *in vitro* against a variety of bacteria; equally active *in vivo* against *M. tuberculosis*. Slightly less effective against other infections.

Toxicity: LD_{50} (mice) 214 mg per kg (no route given). This substance is reputed to be slightly less toxic than dihydrostreptomycin. No vestibular or ototoxic symptoms were observed in human beings treated with the drug.

Utilization: Treatment of tuberculosis.

Reference: 1. Obuchi, S. *et al.* J. Antibiotics (Japan) **11A:** 199–201, 1958.

1,6-Dihydroxyphenazine

Produced by: Streptomyces thioluteus.

Remarks: 1,6-Dihydroxyphenazine is also obtained by the reduction of iodinin, an antibiotic produced by *Pseudomonas iodinum*.

Method of extraction: Culture-filtrate extracted with benzene at pH 6.5 to 7.0 or adjusted to pH 12, filtered, and filtrate neutralized to give a precipitate. Precipitate extracted with benzene. Benzene extracted with 0.1 *N* NaOH, and extract neutralized to give a yellow precipitate. Precipitate re-extracted with benzene. Benzene concentrated *in vacuo*. Crystallized from dioxane or ethyl acetate or purified by sublimation.

Chemical and physical properties: Golden-yellow prisms; m.p. 274°C. Very soluble in dioxane and pyridine. Soluble in alcohols, acetone, ethyl acetate, chloroform, benzene, ethyl ether, aqueous sodium carbonate, and NaOH. Insoluble in water, aqueous sodium bicarbonate, and petroleum ether. Ultraviolet absorption spectrum maxima at 272 ($E^{1\%}_{1cm}$ 6350), 372 ($E^{1\%}_{1cm}$ 245), and 440 to 445 mμ ($E^{1\%}_{1cm}$ 165) (methanol), or 291 ($E^{1\%}_{1cm}$ 4300) and 520 to 530 mμ ($E^{1\%}_{1cm}$ 180) (0.1 *N* NaOH). Infrared absorption spectrum given in reference 1. Purple in alkali. Ethanol solution turns green with $FeCl_3$, gives a blue precipitate with lead acetate. Gives a green-blue precipitate with cupric sulfate and a violet precipitate with silver nitrate. $C_{12}H_8N_2O_2$: C = 68.48%; H = 3.99%; N = 13.51%. Structural formula given in Chapter 6. *Diacetyl derivative:* Light yellow needles; m.p. 235°C.

Biological activity: Active at 3 to 20 μg per ml on certain yeasts and fungi. Not active on bacteria.

Reference: 1. Akabori, H. and Nakamura, M. J. Antibiotics (Japan) **12A:** 17–20, 1959.

DON (6-Diazo-5-oxo-L-norleucine)

Produced by: Streptomyces sp. similar to *S. aureus* and *S. phaeochromogenes* (4).

Method of extraction: Broth adjusted to pH 6.8, filtered, and concentrated *in vacuo*. Concentrate diluted tenfold with 95 per cent ethanol and filtered. Filtrate purified by (a) adsorption on alumina at pH 5.5 to 6.5 and elution with 25 per cent aqueous alcohol; (b) carbon chromatography (1 per cent aqueous acetone as solvent and developer); and (c) crystallization of best carbon fraction from aqueous alcohol or acetone (5).

Chemical and physical properties: Fine light yellow-green needles. Decomposes at 145–155°C with gas evolution. Very soluble in water, aqueous methanol, ethanol, and acetone. Slightly soluble in absolute alcohols. Ultraviolet absorption maxima at 274 ($E^{1\%}_{1cm}$ 683) and 244 mμ ($E^{1\%}_{1cm}$ 376). No spectral shift in alkali or acid. Loss of activity against *Torulopsis albida* concomitant with loss of ultraviolet absorption characteristics in 0.1 *N* alkali or 0.1 *N* HCl (5). Infrared spectrum given in reference 5. $[\alpha]^{26}_{D} = +21°$ (c = 5.4 per cent in water). Positive ninhydrin and Tollen tests. Aqueous solution yields gas when treated with strong acid. Rf values on paper chromatography given in reference 5. Sensitive to extremes of pH and heat. pK_a' values of 2.1 and 8.95 in water. DON has been synthesized and the DL- and D-isomers prepared. C = 42.16%; H = 5.70%; N = 24.07%; diazo N = 16.01%. Molecular weight, 171. $C_6H_9 \cdot N_3O_3$ (2, 5, 14).

Biological activity: Antitumor substance. Slightly active on certain bacteria, yeasts, and *Erro equinus* in eggs. Some activity (at toxic levels) on *Plasmodium lophurae* infections in chicks (4). Unlike azaserine, DON does not give rise to long, nonseptate filaments in *E. coli* (11). Crocker sarcoma 180 growth is "restrained" in mice by oral or intraperitoneal treatment, but the tumor growth potential is not affected (1). DON inhibits Miyono adenocarcinoma, Ehrlich carcinoma, Krebs 2 ascites carcinoma, carcinoma 1025, Ridgway osteogenic sarcoma, Mecca lymphosarcoma, and leukemia L 1210. Slight inhibition of a variety of other carcinomas. No effect on sarcoma T 241, Gardner lymphosarcoma, or Harding-Passey melanoma (10, 13). Active on mast cell neoplasm P 815, but resistant sublines of this tumor can be developed (12). DON is reportedly a better inhibitor of mouse tumors; azaserine is superior in rat tumors (15). Active on an ascitic plasma cell neoplasm (70429) in mice (19).

Toxicity: LD_{50} (mice) 76 ± 14 mg per kg intravenously (4); 250 μg per kg intraperitoneally in a single daily dose for 7 days is tolerated by mice, but with weight loss. Tumor-bearing mice are more susceptible to DON toxicity than normal mice (1). In single doses DON and azaserine have similar toxicity, but DON is 50 to 100 times more toxic in chronic toxicity tests with mice, rats, and dogs (9). LD_{50} of DON in eggs is about $\frac{1}{50}$th that of azaserine. DON or azaserine toxicity to eggs overcome by immediate injection of adenine or hypoxanthine following injection of DON or azaserine (7). Two doses of 0.5 mg per kg given to mother rats between implantation and midterm cause complete litter destruction; the fetus is affected directly, not the placenta, ovaries, or pituitary. This effect can be overcome by adenine. No cumulative toxicity or impairment of fertility was noted in mother rats having repeated complete destruction of litters. Subsequent offspring were normal (17). Toxic symptoms in human beings include oral soreness and ulceration, diarrhea, and vomiting (18).

Utilization: Clinical trials in human beings indicated transient or no activity on a variety of neoplastic diseases (3). Some activity has been reported on Hodgkin's disease (16). Some evidence of "temporary arrest" of neoplastic diseases in human beings (18, 20).

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Duramycin

Produced by: Streptomyces cinnamomeus f. *azacoluta* (1, 3).

Synonym: Fraction B of antibiotic F 17(2). Culture-broths contain three or more antibiotics (3) related to cinnamycin.

Method of extraction: Broth extracted with butanol. Addition of heptane causes separation of an aqueous layer containing most of the antibiotic. Concentration *in vacuo* of watery layer, followed by lyophilization. Purified by chromatography on alumina at pH 4.7 with 80 per cent ethanol as solvent and developer (2).

Chemical and physical properties: Slightly acidic polypeptide. Soluble on heating in absolute methanol, water-methanol, and water-ethanol. Forms a crystalline picrate and alcoholate, and a helianthate. *HCl:* Soluble in water, aqueous acetone, methanol, and ethanol. Slightly soluble in absolute alcohols. Surface-active. Infrared data given in reference 2. No ultraviolet absorption. $[\alpha]_D^{25} = -6.4°$ (c = 3.9 per cent in water). No definite melting point. Positive biuret and azide-iodine tests. Negative $FeCl_3$, Benedict, Molisch, periodic acid, Millon, nitroprusside, Pauly, Sakaguchi, xanthoproteic, and Hopkins-Cole tests. Stable to heat at pH 3 to 9. Acid hydrolysates contain lanthionine, β-methylanthionine, aspartic acid, glycine, glutamic acid, proline, valine, phenylalanine, and possibly ornithine and hydroxyproline. Contains several free carboxyl and at least one free amino group. *Picrate:* m.p. 212–245°C (decomposition). Insoluble in absolute methanol; soluble in water-saturated butanol. C = 51.30%; H = 5.76%; N = 16.85%; S = 3.18% (2).

Biological activity: Active on gram-positive rods; less active on fungi and yeasts (2).

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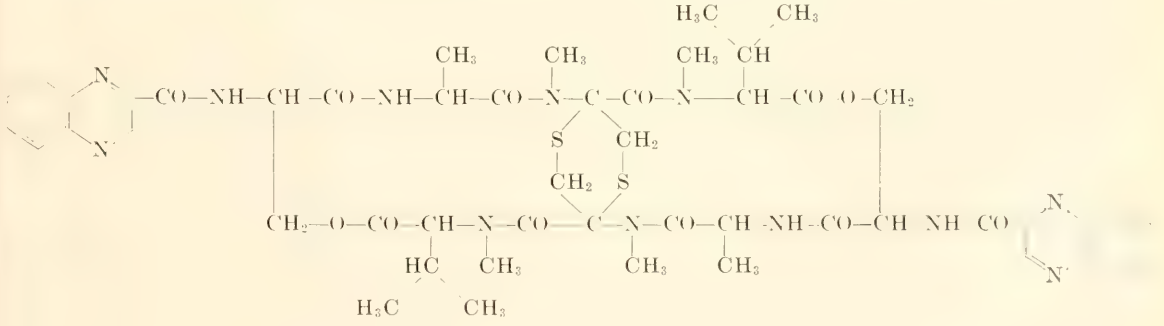
Echinomycin

Produced by: Streptomyces echinatus (2) and an unidentified *Streptomyces* sp. (4).

Synonyms: Antibiotic X 948 (1, 4). May be related to actinoleukin (5).

Method of extraction: Culture-filtrate extracted at pH 7 to 8 with ethyl acetate; extract freed from inactive bases and acids and concentrated *in vacuo*. Active substance precipitated with petroleum ether and purified by chromatography over alumina. Crystallization from methyl alcohol (3).

Chemical and physical properties: Weakly basic (3). Colorless powder, m.p. 217–218°C (3); or colorless prisms, m.p. 236–238°C (4). Soluble in 20 per cent HCl, but not in other dilute mineral acids. Ultraviolet absorption spectrum maximum 243 mμ (log ε = 3.81). Infrared spectrum given in reference 3. $[\alpha]_D = -31C°$ (c = 0.86 per cent in $CHCl_3$) (3). Negative tests for thio- and disulfide groups (6). $C_{29}H_{37}O_7N_7S$ (3, 4) or $C_{50\text{-}52}H_{60\text{-}64}O_{12}N_{12}S_2$: C = 55.45%; H = 5.88%; N = 15.33%; S = 5.27%; N—CH_3 = 5.2%; C—CH_3 = 2.75%. Molecular weight, 1050 ± 50 or 1604 ± 30 (6). Acid hydrolysis products include D-serine, L-alanine, L-N-methyl valine, and N-methyl-N′-phenylthiourea. Alkaline hydrolysis yields quinoxaline-2-carboxylic acid (3, 6). Probable structural formula (6):



Biological activity: Active against gram-positive, gram-negative, and acid-fast bacteria, protozoa, and viruses. Some cancerolytic activity. Active *in vivo* against *Trypanosoma equiperdum* and *T. brucei* infections in mice (CD_{50} about 0.36 mg per kg subcutaneously). Local antitrichomonal activity (1).

Toxicity: Toxic to young chicks at 0.01 per cent in diet (4). LD_{50} (mice) 3.8 mg per kg subcutaneously, 0.75 mg per kg intra-abdominally, >2500 mg per kg orally (1), 0.4 mg per kg intraperitoneally (2).

References:

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Echinomycin-like Antibiotic

Produced by: Streptomyces sp. (1) differing from echinomycin-producer.

Synonym: Antibiotic X 1008 (1).

Method of extraction: Whole broth extracted with butanol. Extract concentrated *in vacuo*. Antibiotic precipitated with petroleum ether. Successively extracted into methylene chloride and methanol, then crystallized from an ethanol-acetonitrile mixture (1).

Chemical and physical properties: Cube-like crystals; m.p. 209–216°C (decomposition). $[\alpha]_D^{27} = -282°$ (c = 1 per cent in chloroform). Gives a melting point depression when mixed with echinomycin. Same ultraviolet spectrum as echinomycin. Infrared spectrum differs in certain details from that of echinomycin. $C_{29}H_{35}O_7N_6S$: C = 56.40%; H = 6.52%; N = 13.69%; S = 5.08%. The side chain attached to the quinoxaline residue probably differs from echinomycin (1).

Biological activity: Same *in vitro* antibacterial activity as echinomycin. No trypanocidal activity in mice. Only partial cross-resistance with echinomycin.

Toxicity: Highly toxic (1).

Reference: 1. Berger, J. *et al.* Experientia **13:** 434–436, 1957.

Ehrlichin

Produced by: Streptomyces lavendulae.

Method of extraction: Culture-filtrate adjusted to pH 2.0 with concentrated HCl. A dark brown precipitate collected by centrifugation.

Chemical and physical properties: Stable at neutrality and alkaline pH. Nondialyzable. Inactivated *in vitro* by horse serum. Unaffected by tryptic digestion.

Biological activity: Inhibitory to influenza A and influenza B *in vitro*. Active *in vivo* against influenza B. Inactive against bacteria, fungi, *Chlamydozoaceae*, pox viruses, and bacterial viruses.

Toxicity: LD_0 (mice) 100 mg per kg intraperitoneally, 300 mg per kg subcutaneously.

Reference: 1. Groupé, V. *et al.* J. Immunol. **67:** 471–482, 1951.

Elaiomycin

Produced by: Streptomyces gelaticus (4) initially identified as *S. hepaticus* (1, 3); *Streptomyces* sp. (5).

Synonym: Identical to or closely related to hygroscopin A.

Method of extraction: I. Broth-filtrate extracted with ethyl acetate at pH 7.0. Extract concentrated *in vacuo*. Residue successively extracted with water. Extract filtered, and extracted with petroleum ether at pH 7.0. Solvent removed *in vacuo* and residual oil extracted with ether. Ether distilled off and oily residue subjected to molecular distillation at pressures of $<1\ \mu$ at 60–61°C. Purified by countercurrent distribution (n-heptane-methanol-water containing 0.125 per cent (by weight) Na_2SO_4 ; 1.25:1.25:1). Active fractions concentrated *in vacuo* at 37°C. Residue saturated with NaCl and extracted with n-heptane. Extract concentrated *in vacuo* and residue subjected to molecular distillation to give elaiomycin. II. Residue from ethyl acetate-extract of broth (see I) extracted with n-heptane. Upper phase solvent removed by evaporation *in vacuo*. Residue purified by chromatography on HCl-washed alumina adjusted to pH 4.5, using multiple absorption columns in series, and n-heptane as solvent and developer. Eluted with ether or methanol. Oily residue after removal of solvent from active fractions is subjected to molecular distillation (1, 3).

Chemical and physical properties: Slightly yellow oil. Soluble in all common organic solvents but sparingly soluble in water. Ultraviolet absorption spectrum maximum (methanol) 237.5 mμ (ϵ = 11,000), remaining unchanged in polar and non-

polar solvents and at acidic and alkaline pH in aqueous solution. Peak gradually disappears in 0.1 *N* NaOH. Infrared spectrum given in reference 1. $[\alpha]_D^{26} = +38.4°$ (c = 2.8 per cent in absolute ethanol). Gives a deep purple color when placed in light having a wave length of 256 mμ. $n_D^{25} = 1.4798$. Positive iodoform test. Negative $FeCl_3$, Benedict, ninhydrin, Sakaguchi, periodic acid, xanthate (for OH^- groups), hydroxamate (ester groups), sodium nitroprusside (for methyl ketone), and Ehrlich tests. Stable in neutral or acidic aqueous solutions, but in 0.1 *N* NaOH decomposes into a yellow product of unknown character. $C_{13}H_{26}N_2O_3$: C = 60.12%; H = 10.06%; N = 10.95%. Molecular weight, 244. Acid hydrolysis products include racemic α-hydroxyoctanoic acid. Contains an aliphatic α,β-unsaturated azoxy group and exists in the D-threo configuration (1, 3, 6). Structural formula is given in Chapter 6.

Biological activity: Active on human and bovine varieties of *M. tuberculosis* but not on other types of mycobacteria. Very slight activity on fungi. Not active on bacteria. Not active *in vivo* (mice and guinea pigs) on tuberculosis (2).

Toxicity: LD_{50} (mice) 43.7 mg per kg intravenously, 62.5 mg per kg subcutaneously (2).

References:

1. Haskell, T. H. *et al.* Antibiotics & Chemotherapy **4:** 141–144, 1954.
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4. Anderson, L. E. *et al.* Antibiotics & Chemotherapy **6:** 100–115, 1956.
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Elaiophylin

Produced by: Streptomyces melanosporus var. *melanosporofaciens*. The same organism produces melanosporin.

Synonym: Similar to azalomycin B.

Method of extraction: See melanosporin.

Chemical and physical properties: White crystals; m.p. 178–183°C (decomposition). $[\alpha]_D^{20} = -49°$ (in chloroform). Soluble in chloroform, acetone, and ethyl acetate. Slightly soluble in lower alcohols. Insoluble in water, ether, and benzene. Tentative empirical formula: $(C_6H_{10}O_2)_n$. Light-absorption maximum at 252 mμ. Infrared absorption spectrum given in reference 1.

Biological activity: Active against gram-positive bacteria.

Toxicity: Mice tolerate 100 mg per kg intraperitoneally.

Reference: 1. Arcamone, F. M. *et al.* Giorn. microbiol. **7:** 207–216, 1959.

Endomycins

Produced by: Streptomyces endus (closely related to *S. hygroscopicus*) (7, 12), and *Streptomyces* sp. (2, 13).

Synonyms: Helixins A and B. Related to mediocidin. A second, nonpolyenic, ether-soluble antibiotic (9-20F-1) present in endomycin broths may be the same as helixin C (2, 13).

Method of extraction: Mycelium and precipitate from acidified broth extracted successively with butanol. Extract evaporated with addition of water. Residue treated with ether and boiling benzene, then suspended in water and retreated with ether. Aqueous layer (pH 8.5 to 9.0) centrifuged. Supernatant acidified to precipitate dark brown gummy antibiotic. Taken up in 0.05 *N* NaOH and precipitated with glacial acetic acid; or, taken up in absolute alcohol and reprecipitated with amyl acetate (2, 8). May also be extracted in the same way as candidin and separated into components A and B by countercurrent distribution (ethyl acetate-n-propanol-0.1 *N* aqueous NH_4Ac, 3:1:3) (13).

Chemical and physical properties: Complex: Yellow-brown powder. Soluble in alcohols containing 10 to 20 per cent water, methyl Cellosolve, dimethylformamide, pyridine, glacial acetic acid, 0.2 *N* NaOH, or HCl. Soluble in water at pH 2.0 or below and at pH 7.0 or above; insoluble in water at pH 4 to 6. Partially soluble in methanol and ethanol; sparingly soluble in dioxane. Insoluble in ether, chloroform, benzene, ethyl acetate, acetone, and other nonpolar solvents. Can be precipitated from aqueous solution by Na^+, Ca^{++}, or Mg^{++}. Surface-active and forms emulsions easily. Slowly diffusible. Low nitrogen content (about 3.7 per cent). Stable to acid and alkali and to autoclaving at pH 7.0. $pK_a^1 = 2.5$; $pK_a^2 = 5.5$ to 6.5; $pK_a^3 = 9$. Contains two major components: A, a tetraene; and B, a hexaene. A greater amount of the tetraene was present in the helixin complex than in the endomycin complex. The helixin D component is present in varying quantities in endomycin. Rf values for A and B are 0.04 and 0.37 (water-saturated n-butanol), 0.09 and 0.38 (water-saturated n-butanol on paper buffered with 0.1 *N* sodium phosphate at pH 12.0), and

0.30 and 0.70 (t-butanol-water, 4:1). *Endomycin A:* Ultraviolet absorption spectrum maxima at about 292, 308, and 320 mμ. *Endomycin B:* Ultraviolet absorption spectrum maxima at 338, 359, and 380 mμ (13).

Biological activity: Active on fungi, bacteria, and *Trypanosoma cruzi*. More active on yeast-like fungi (0.25 to 10 μg per ml) than on filamentous fungi (10 to 13 μg per ml). More active on gram-positive than on gram-negative bacteria. Some activity on *T. cruzi* infections in mice (2, 4). Active (not fungicidal) on *C. albicans* in tissue culture and lytic to *Trichomonas vaginalis in vitro* (5, 6). Controls leaf rust of wheat (3), strawberry fruit rot (*Botrytis cinerea*) (10), and turf brown patch (*Rhizoctonia solani*) (9); provides partial protection against *Pseudoperonospora cubensis* (11). Some control of bean root rot (14) and downy mildew of broccoli (*Peronospora parasitica* (15)). Antifungal activity enhanced by the neomycins (16).

Toxicity: Mice tolerate 0.5 gm per kg but not 1 gm per kg (no route given) (2). The LID (least injurious dose) to tissue cultures of chick heart, spleen cells, and human skin are 100 to 200 μg per ml, 100 to 200 μg per ml, and 50 to 100 μg per ml, respectively (5, 6). Produces some abnormalities in *Allium cepa* roots at 100 ppm (1). Nontoxic as 10,000-ppm spray to wheat, tomato, or bean plants (3).

Utilization: Control of plant diseases.

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Endomycin-like Complex, Helixins

Produced by: Streptomyces sp. (1).

Method of extraction: Precipitate formed in filtered broth at pH 3.0; extracted with ethanol. Extract concentrated *in vacuo*; helixin precipitates out as a red gum on addition of chloroform (1). Gum dissolved in absolute ethanol, and water added. Mixture filtered and extracted with ethylene dichloride to remove helixin C. Aqueous ethanol layer concentrated under reduced pressure and extracted with diethyl ether following addition of $NaHCO_3$. Aqueous layer acidified to pH 3.0 and precipitate filtered off and taken up in absolute ethanol. Ethanol solution extracted with ethyl acetate and filtered. Filtrate dried *in vacuo*. By dissolving this preparation in 0.06 N NH_4OH and extracting with 1:1 n-butanol-ethyl acetate, helixin b is obtained, contaminated with a small amount of helixin D. The aqueous layer remaining is extracted with n-butanol. Concentration of this extract to dryness gives helixin A. Helixin B is further purified by partition chromatography (3).

Chemical and physical properties: Helixin complex: Contains four components, A, B, C, and D, having approximate Rf values of 0.07, 0.42, 0.85, and 0.68, respectively (butanol-ethyl acetate, 1:1) (3). Soluble in ethanol, methanol, pyridine, and glacial acetic acid. Slightly soluble in n-butanol, acetone, and chloroform. Insoluble in ether, petroleum ether, benzene, ethyl acetate, and carbon tetrachloride. Negative Molisch, ninhydrin, Hopkins-Cole, xanthoproteic, Millon, and $FeCl_3$ tests. More stable at alkaline pH; somewhat less stable than endomycin (1). Most active at alkaline pH (2). May have one or more components in common with endomycin (3).

Biological activity: Active against yeasts and fungi at 15 μg per ml or less. Active on bacteria at >30 μg per ml (1). Control of *Helminthosporium victoriae* blight of oats and *H. sativum* seeding blight of barley with seed treatment (greenhouse tests). In the field, seed treatment with helixin B controls wheat bunt, oat smut, and covered smut of barley (4). Protective action against tomato early blight (*Alternaria solani*) (2).

Toxicity: Helixin B toxic to tomato and cowpea cuttings at 7.5 to 15 μg per ml. Not toxic to whole tomato plants at 90 μg per ml when used as a watering solution. No phytotoxic effects at 3 mg per ml, applied as a spray on young bean, corn, cowpea, tomato, and wheat plants. Inhibition of seed germination at 25 to 100 μg per ml (2).

Utilization: Plant diseases caused by fungi.

References:

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3. Smeby, R. R. *et al.* Phytopathology **42:** 506–511, 1952.
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Enteromycin

Produced by: Streptomyces albireticuli (1, 3). This culture also produces eurocidin, carbomycin, and tertiomycin A (5).

Method of extraction: Extracted from culture-broth with ethyl acetate at pH 2.0. Back-extracted into Na_2CO_3. Re-extracted into ethyl acetate at acid pH (acidified with H_2SO_4). Recrystallized from ethanol (2).

Chemical and physical properties: Acidic substance. Pale yellow crystals. Decomposes at 160–162°C. Soluble in methanol, ethanol, ethyl acetate, butyl acetate, and dioxane. Sparingly soluble in water, acetone, and chloroform. Insoluble in benzene, ether, and petroleum ether. Ultraviolet absorption spectrum maximum at 300 to 320 mμ (ethanol). Optically inactive in methanol. Contains carbonyl and C—OCH_3 groups. C = 38.21%; H = 4.62%; N = 14.32%. $C_6H_8O_5N_2$. Releases CO_2 on addition of sodium bicarbonate (2–4).

Biological activity: Active on gram-negative bacteria, including *E. coli*, *Proteus*, *Serratia*, *Sal. typhi*, *Shigella*, *V. cholerae*, and *Pseudomonas*. Less active on gram-positive bacteria, including *Staph. aureus*, *B. subtilis*, *Ps. aeruginosa*, and mycobacteria. Active on certain viruses. Not active on fungi. More active at acid than alkaline pH (2–4).

Toxicity: LD_{50} (mice) 135 to 138 mg per kg intravenously (2, 4).

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Erythromycin

Produced by: Streptomyces erythreus (13).

Synonym: Erythromycin A.

Method of extraction: I. Broth-filtrate extracted with amyl acetate or methyl isobutyl ketone at pH 9.4. Back-extracted into water at pH 5.1. Aqueous extract adjusted to pH 8.0, concentrated, and readjusted to pH 9.5 to 11.0 to precipitate erythromycin. Recrystallized from acetone-water or petroleum ether (9, 13). II. Broth-filtrate defatted with petroleum ether, adjusted to pH 8.5, and extracted with ethyl acetate or butanol. Extracts evaporated to dryness *in vacuo*. Solid triturated with petroleum ether. Precipitated from ethanol on addition of water and cooling. Recrystallized from ethanol and aqueous acetone to give the "erythromycin acid addition salt." Also isolated by absorption on acid-treated charcoal and butanol elution. "Acid addition salt" suspended in water, pH adjusted to 6.3, and washed with $CHCl_3$. Aqueous layer extracted with amyl acetate at pH 9.8. Extracts evaporated off *in vacuo*. Residue recrystallized from aqueous ethanol (13). III. Crude powders, containing two or more components of the complex, yield, on dissolving in nitromethane, warming, decolorizing, and cooling, essentially pure erythromycin, leaving the other components in the mother liquor. Recrystallized from the same solvent and aqueous acetone (37). IV. Crude erythromycin salt solution adjusted to pH 8.0 to 8.5, heated to 35–45°C, stirred with acetone, ethanol, or isopropanol and a water-soluble salting-out agent such as NaCl. The whole adjusted to pH 10 to 10.8, and heated to 35–45°C to bring erythromycin into the organic phase. Water added to turbidity at 45°C and the whole cooled to 15°C to give crystals (38).

Chemical and physical properties: Basic macrolide (1). *Free base* (hydrate or solvates from acetone or chloroform): White needles of the hexagonal system or short rods; m.p. 136–140°C or 134–136°C (15). If slow heating is continued, resolidifies, then melts again at 190–193°C (15). Very soluble in alcohols, acetone, chloroform, acetonitrile, and ethyl acetate. Moderately soluble in ether, ethylene dichloride, and amyl acetate. Soluble to 2 mg per ml in water (1, 13). Ultraviolet absorption spectrum maximum at 278 mμ (ϵ = 27) (15), 280 mμ (ϵ = 50, broad peak, pH 6.3) (1), or 288 to 289 mμ ($E^{1\%}_{1cm}$ 0.395) (13). Infrared spectrum given in references 13 and 15. $[\alpha]_D^{25} = -78°$ (c = 1.99 per cent in ethanol) (1, 38). Separation from

erythomycin B by a variety of systems with paper chromatography has been reported (18). Other information on Rf values given in reference 13. $pK_a' = 8.6$ to 8.8 (1, 15). Crystallographic data given in references 1, 13, and 16. Moderately stable at pH 5.0 to 8.5; 75 per cent activity lost on boiling for 1 hour; 50% lost after heating at 60°C for 5 minutes (3). C = 61.05%; H = 9.43%; N = 1.91%; C—CH_3 = 17.98% (13). After drastic acid degradation, products include dimethylamine and desosamine. *Desosamine* (3-dimethylamino-4-desoxy-5-methylaldopentose) (I): $C_8H_{17}NO_3HCl$; m.p. 183–184°C or 191–193°C. $[\alpha]_D^{25} = +54.5°$ (c = 2 per cent in ethanol) (9, 15). Treatment with 0.3 *N* HCl at 25°C yields a nitrogen-free sugar, cladinose (II), a neutral oil, $C_8H_{16}O_4$, and erythralosamine (III). *Cladinose:* Soluble in water, alcohols, acetone, ether, and benzene; slightly soluble in petroleum ether. Positive Tollen and iodoform tests. Acid-labile (10, 25). *Erythralosamine:* Elongated prisms; m.p. 206–207°C. Soluble in dilute HCl, alcohols, acetone, ether, chloroform, and benzene. Insoluble in water and dilute alkali. Hydrolysis products include I (10, 15). Reduction of erythromycin gives *dihydroerythromycin*, m.p. 133–135°C, $C_{37}H_{69}NO_{13}$ (IV). Treatment with HCl-methanol removed II, to give a substituted polyhydroxylactone, $C_{29}H_{55}NO_{10}$ (V), differing from III. Acid hydrolysis of V gave *dihydroerythronolide* (VI), $C_{21}H_{40}O_8$, in which the ketone group of the aglycone of erythromycin (*erythronolide*) has been reduced to a hydroxyl (24, 26). Structural formula ($C_{37}H_{67}O_{13}N$) given in Chapter 6. *Solvates:* Very stable to heating. Solvent easily removed on addition of water (39). *Hydrochloride:* White needles; m.p. 170–173°C. Very soluble in lower alcohols. Soluble to 40 mg per ml in water. Slightly soluble in ethyl acetate, ether, amyl acetate, and chloroform (5, 13). Infrared spectrum given in reference 13. *Acid addition salt* (with unidentified organic acid, see II under "Methods of Extraction"): White hexagonal needles; m.p. 82–83.5°C (13). A number of derivatives, none of which had activity greater than erythromycin, are reported in references 31 and 32.

Biological activity: In vitro: Active on gram-positive bacteria, mycobacteria, corynebacteria, *Actinomyces israelii*, clostridia, and certain gram-negative bacteria of the *Hemophilus-Brucella* group (1–3, 6, 8). Not active on *N. asteroides* or pathogenic fungi (8). Active on *Endamoeba histolytica* only in the presence of bacteria, and *Trichomonas vaginalis* (1, 12). Active on pleuropneumonia-like organisms isolated from arthritic goats (27). Most active at alkaline pH (3). Bacteriostatic or bactericidal depending on concentration of the antibiotic and on the organism used. Active only on multiplying bacteria (4). Cross-resistance with carbomycin, spiramycin, oleandomycin, and streptogramin (22). *In vivo:* Active (mice) on *Streptococcus pyogenes*, *D. pneumoniae*, *Hemophilus pertussis* (only when organism was injected intranasally, not intracerebrally), *Corynebacterium diphtheriae*, *Clostridium tetani*, *Borrelia novyi*, *Leptospira icterohaemorrhagiae* (hamsters), and moderately active on *M. tuberculosis* (1, 6, 7, 14, 29, 30). Active on *Endamoeba histolytica* (rats) (12), *Trichomonas vaginalis* (1), *Trypanosoma equiperdum* (but not *T. cruzi*), and toxoplasmosis in mice (1, 12). Active (chick embryos) on *Rickettsia prowazekii* and *R. typhi*; moderately active on *R. rickettsii* and *R. akari*; and slightly active on *Coxiella burnetii* (7, 20). Active (chick embryos) on psittacosis, meningopneumonitis, lymphogranuloma venereum, and feline and mouse pneumonitis agents (1, 6, 7, 21, 28), but not on MM, Semiliki Forest, poliomyelitis (Lansing type 2), influenza A (PR 8), or lymphocytic choriomeningitis viruses (7). Active (mice) on oxyurids, *Syphacia obvelata*, and *Aspicularis tetraptera* (12). Increases growth rate of swine, chicks (11), and turkey poults (36).

Toxicity: Base: LD_{50} (mice) 1800 to >2500 mg per kg subcutaneously, 3112 ± 211 mg per kg orally, and >700 mg per kg intraperitoneally (1, 5). LD_{50} (rat) >3000 mg per kg orally, >2000 mg per kg subcutaneously (5). LD_{50} (guinea pig) 413.4 ± 51.7 mg per kg intraperitoneally (5). A delayed toxicity for guinea pigs was reported (17). Pigs exhibiting toxic symptoms at as low a dose as 1.5 mg per pig have no gross morphological findings except paleness and spleen shrinkage (35). LD_{50} (hamster) 3018 ± 190 mg per kg orally (5). Increasing toxicity to hamsters was noted over a period following introduction of the drug into the laboratory (30). *Hydrochloride:* LD_{50} (mice) 425.6 ± 15.7 mg per kg intravenously, 490.6 ± 30.4 mg per kg intraperitoneally, 1849 ± 89 mg per kg subcutaneously, and 2927 ± 162 mg per kg orally (5). Nontoxic to carnation cuttings at 120 ppm (23). Least injurious doses for human skin and chick embryo spleen cells in tissue culture are 85 to 170 and 30 to 60 μg per ml, respectively (19). Highest concentration permitting migration of epithelial cells in tissue culture is 2.5 mg per ml (40). Minimal dose inhibiting mitosis of HeLa cells is 1 to 2 mg per ml (33).

Utilization: Infections caused by gram-positive bacteria, especially those in which penicillin can-

not be used because of sensitivity of the patient or resistance of the organism. Amoebiasis.

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Erythromycin B

Produced by: Streptomyces erythreus.

Synonym: Closely related to erythromycin.

Remarks: The proportions of the erythromycins produced can be varied by changing the nitrogen content of the culture media (5).

Method of extraction: I. Broth at pH 9.5 extracted with chloroform or amyl acetate, then back-extracted into 0.1 *M* phosphate buffer at pH 5.2. Extraction procedures repeated. Purified and separated from erythromycin by column chromatography on powdered cellulose with 0.01 *N* NH_4OH, saturated with methyl isobutyl ketone as developer and eluant, or by countercurrent distribution (acetone-methyl isobutyl ketone-0.1 *N* phosphate buffer, pH 6.5, 1:20:20). Active fractions concentrated *in vacuo*; extracted from the aqueous solution with chloroform at pH 9.6 to 10.5. Chloroform evaporated off, residue extracted with ether, again evaporated, and the antibiotic crystallized from acetone (1, 2). II. Amyl acetate extract of broth back-extracted into aqueous acetic acid at pH 5.0 to 6.5 and traces of amyl

acetate removed by distillation *in vacuo*. Salted into acetone at pH 10 and precipitated from acetone by addition of water. Crystallized from acetone as a mixture of A and B. Mixture dissolved in an aqueous solvent at pH 1.4. Standing at this pH for 40 minutes destroys A. B is precipitated on addition of NaOH, crystallized from water-acetone, and recrystallized from dry acetone (7).

Chemical and physical properties: Basic substance. Rectangular plates; m.p. 198°C or 201–203°C (uncorrected). Soluble in ether, acetone, chloroform, ethyl acetate, and benzene. Sparingly soluble in water. Ultraviolet absorption spectrum maximum at 289 mμ (E = 36.4). Infrared spectrum given in reference 2. $[\alpha]_D^{25} = -78°$ (c = 2 per cent in ethanol). $pK_a' = 8.8$. Molecular weight, 730. More stable to acid than erythromycin, a property which permits their separation. Rf = 0.6 (methanol-acetone-water, 19:6:75); erythromycin Rf = 0.7. Water-soluble acid salts: *Hydrochloride*, m.p. 149–150°C; *Stearate*, m.p. 54–57°C. Base: $C_{37}H_{67}NO_{12}$: C = 62.08%; H = 9.56%; N = 1.99%; C—CH_3 = 14.61%; O—CH_3 = 4.79%. Mild acid hydrolysis products include: neutral oil, cladinose, $C_8H_{16}O_4$, and two crystalline bases, one optically active, $C_{29}H_{55}NO_9$ (m.p. 119–121°C), and the other optically inactive, $C_{29}H_{51}NO_8$ (m.p. 239–240°C). Strong acid hydrolysis produces desosamine, $C_8H_{17}NO_3$. Structural formula of erythromycin B (1, 2, 4, 6, 7) given in Chapter 6.

Biological activity: Qualitatively, B has the same antimicrobial activity as erythromycin, but is only 75 to 85 per cent as active quantitatively. Cross-resistance exists between A and B. Resistance to B develops more readily than to A (2, 5).

Toxicity: Twice as toxic as erythromycin (3).

References:

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7. Denison, F. W. *et al.* U. S. Patent 2,834,714, May 13, 1958.

Erythromycin C

Produced by: Streptomyces erythreus. This culture also produces erythomycins A and B.

Method of extraction: Broth-filtrate extracted with chloroform at pH 9.75. Extracts concentrated *in vacuo* and chilled to precipitate erythromycin and erythromycin B. Supernatant dried *in vacuo*, dissolved in upper phase of an equilibrated methyl isobutyl ketone-0.1 N sodium phosphate buffer (pH 6.5)-acetone system (20:20:1) and subjected to countercurrent distribution to separate C from erythromycin. Active fractions containing C adjusted to pH 9.75 and extracted with chloroform. Extract dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo* to precipitate C.

Chemical and physical properties: Basic substance. Needle-shaped crystals; m.p. 121–125°C. Can be separated from erythromycin by chromatography on cellulose, developed with 0.01 N NH_4OH saturated with methyl isobutyl alcohol. Soluble in chloroform, acetone, and ether. Relatively insoluble in water. $pK_a' = 8.5$. Ultraviolet absorption spectrum maximum at 292 mμ (E = 108). Infrared spectrum given in reference 1. $C_{36}H_{65}NO_{13}$: C = 59.75%; H = 9.10%; N = 1.95%; O = 29.49%. Molecular weight, 730. Acid methanolysis yields erythralosamine and a neutral sugar, $C_7H_{14}O_4$. Although this formula is the same as that of mycarose from carbomycin, the two sugars are not the same. Erythromycin C differs from erythromycin by the absence of the methoxyl group in cladinose (see Chapter 6).

Biological activity: Similar to erythromycin and erythromycin B.

Reference: 1. Wiley, P. F. *et al.* J. Am. Chem. Soc. **79:** 6074–6077, 1957.

Etamycin (Viridogrisein)

Produced by: Streptomyces sp. resembling *S. lavendulae* (1), *S. griseus* (2, 5), and *S. griseoviridus* (5).

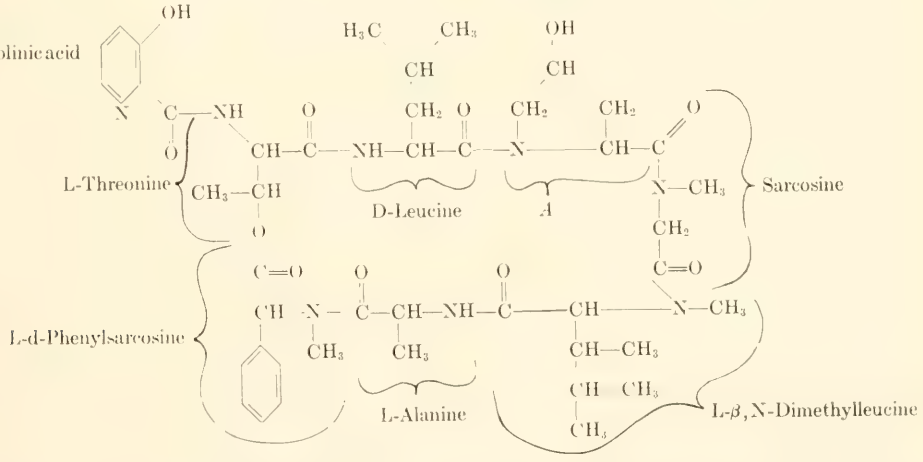
Synonyms: Antibiotic K 179 (11), viridogrisein. Possible synonym: antibiotic 6613 (12, 13).

Remarks: Etamycin contains four amino acids not previously found in nature (9).

Method of extraction: I. Broth extracted with methyl isobutyl ketone or ethylene dichloride. Extract concentrated and: (a) concentrate added to 10 volumes of Skellysolve B, which forms a precipitate of crude etamycin. Crude etamycin dissolved in acetone and precipitated as the hydrochloride; or (b) chromatographed on alumina adjusted to pH 5.0 to 7.0 with HCl. Eluted with 50 per cent ethyl acetate, 40 per cent methanol, and 10 per cent water. Purification by countercurrent distribution (benzene-methanol-water-n-heptane-$(Na)_2SO_4$, 7:10:6:6:0.125 by weight with respect to water) (1, 2). II. Precipitates almost quanti-

tatively from solutions of ethanolic ammonia as the ammonium salt, leaving almost all impurities in solution. This colorless, crystalline salt is unstable in the dry state and decomposes to the pure antibiotic *in vacuo* (10).

Chemical and physical properties: Macrocyclic peptide lactone, containing eight amino acids. Weakly basic (1) or amphoteric (3). *Base:* Colorless amorphous powder (10). Soluble in lower alcohols and ketone, benzene, chloroform, carbon tetrachloride, carbon disulfide, ethyl acetate, ether, 1 *N* HCl, and 1 *N* NaOH. Slightly soluble in water. Insoluble in petroleum ether (1, 9). $\lambda_{max}^{H_2O}$ 350 ($E_{1cm}^{1\%}$ 71) with a shoulder at 303 ($E_{1cm}^{1\%}$ 34) (3). $[\alpha]_D^{25} = +7.7°$ (c = 2 per cent in methanol) (1). Positive tests for an active methylene group, peptide bond, ester, and amide groupings (hydroxamic acid test). Brownish red color with $FeCl_3$ test. Weakly positive Folin-Ciocalteau and Bayer tests. Negative ninhydrin and Sakaguchi tests (1, 3). $pK_a' = 7.4$ (10 per cent aqueous ethanol). Stable at acid pH and neutrality; inactivated by alkali. Esterification destroys microbiological activity. Calculated formula: $C_{44}H_{62}O_{11}N_8 \cdot H_2O$: C = 59.0%; H = 7.05%; N = 12.3%; N—Me = 5.35%. Molecular weight, 800 to 900. Proposed structure (9):



Hydrochloride: m.p. 163–170°C (decomposition). Soluble in methanol, ethanol, formamide, and in water to the extent of 4 mg per ml. Slightly soluble in acetone, ethyl acetate, and less polar solvents. λ_{max}^{MeOH} 304 ($E_{1cm}^{1\%}$ 86) with strong end-absorption at <250 (1).

Biological activity: Active *in vitro* against gram-positive bacteria, including clostridia, at a concentration of 0.04 to 2.5 μg per ml. Active on *Endamoeba histolytica*. Inactive against most gram-negative bacteria and *C. albicans*. Active *in vivo* against experimental *D. pneumoniae* and *Staph. aureus* infections in mice and *E. histolytica* infections in rats and dogs. Some antirickettsial activity; no antiviral activity. Active against bovine mastitis, infectious bronchitis of chicks, and panleukemia of cats (1, 4, 6). Active *in ovo* on pleuropneumonia-like organisms (avian) (7). Intermediate activity on sarcoma 180 in mice (8).

Toxicity: LD_{50} (mice) 273.5 mg per kg intraperitoneally, >2000 mg per kg subcutaneously, >3000 mg per kg orally. Etamycin produces a reversible leukopenia in dogs (1). At certain concentrations, given orally or subcutaneously to mice, nonedematous weight gain without increased food intake was noted (4).

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Etruscomycin

Produced by: Streptomyces lucensis (1, 2).

Synonym: Antibiotic 1163 F. I. (2).

Method of extraction: Mycelium and culture-filtrate extracted with n-butanol, methanol, ethanol, or isopropyl alcohol. Extract concentrated *in vacuo* to give an active precipitate. Ether added to the mother liquors precipitates a further active fraction. Purified by washing with acetone, followed by hot aqueous isopropanol; taking up in anhydrous methanol containing $CaCl_2$. Precipitated as the base on addition of water. Chromatographed on silica gel. Crystallized from water saturated with butanol (1, 3).

Chemical and physical properties: Amphoteric conjugated tetraene. White crystals. Browns, gradually followed by decomposition at >150°C. Soluble in dimethylformamide, pyridine, glacial acetic acid, and by the formation of salts in acidic or alkaline methanol. Moderately soluble in aqueous lower alcohols; slightly soluble in anhydrous methanol and water; insoluble in acetone, chloroform, ether, benzene, and other nonpolar solvents. Ultraviolet absorption spectrum maxima at 290, 305, and 317 mμ ($E^{1\%}_{1cm}$ about 840, 1385, and 1170). Infrared spectrum given in reference 3. $[\alpha]_D^{20}$ = +49.8° (in methanol containing 0.1 *N* HCl); +296° (pyridine). Gives a red-brown color with sulfuric acid. Positive $KMnO_4$ and bromine in carbon tetrachloride tests. Negative $FeCl_3$ and Molisch (weak brown) tests. Stable in the dry state. Labile to heat, light, air, alkali, and acid. Most stable in aqueous solution at pH 7.0 (3). Rf values in various systems of paper chromatography given in reference 3. Broth was reported to contain three components, A, B, and C, all differing in their physicochemical characteristics (2).

Biological activity: Moderately active on yeasts, fungi, *Trichomonas vaginalis*, and *Endamoeba histolytica*. Active *in vivo* (mice) on intestinal *Candida* infections. Also active (rats) on *Endamoeba muris* and intestinal flagellates. Not active on bacteria (1).

Toxicity: LD_{50} (mice) 44.6 mg per kg intravenously, 37.1 mg per kg intraperitoneally, and 1263 mg per kg orally (1).

References:

1. Arcamone, F. *et al.* Giorn. microbiol. 4: 119–128, 1957.
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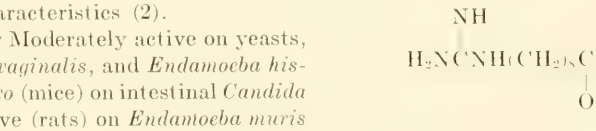
3. Arcamone, F. and Perego, M. Ann. chim. (Rome) 49: 345–351, 1959.

Eulicin

Produced by: Streptomyces sp. closely related to *S. parvus*. This organism also produces an actinomycin and a basic antibiotic which is active against gram-positive and gram-negative bacteria.

Method of extraction: I. Adsorption on charcoal, followed by elution with acidic alcohol. II. Adsorption on cation exchange resins and elution with acid. III. Precipitation of antibiotic from culture-filtrate as the insoluble picrate. The crude picrate extracted with methanol; addition of hydrochloric acid and ether to the methanolic extract precipitates eulicin hydrochloride. The crude hydrochloride is passed through a column of Duolite S-30 resin. Effluent from the column is titrated with a solution of methyl orange, resulting in the precipitation of the insoluble helianthate. The helianthate is crystallized and recrystallized from 80 per cent ethanol. Eulicin hydrochloride is obtained by the addition of an excess of hydrochloric acid to methanolic solutions of the helianthate. The helianthic acid precipitate is removed by filtration, and eulicin hydrochloride is precipitated by addition of ether.

Chemical and physical properties: Basic substance. *HCl salt:* White hygroscopic powder. Poorly defined infrared spectrum. *Helianthate:* Sinters at 142°C; m.p. 154–156°C (decomposition). *Free base:* Hygroscopic gummy substance decomposing with release of ammonia within a few hours of preparation. Weakly dextrorotatory. Positive Sakaguchi test. Alkaline hydrolysis products include 9-aminononanoic acid. Acid hydrolysis products include 9-guanidinononanoic acid and "eulicinine," the latter prepared as the helianthate; m.p. 155–158°C. Structure of eulicinine (1, 4):



The structure of eulicin ($C_{24}H_{52}O_2N_8$) is given in Chapter 6.

Biological activity: Active *in vitro* on yeasts and fungi at 0.018 to 5.0 μg per ml, but only very slightly active (121 μg per ml) on *C. albicans*. Active on *N. asteroides* at 2.3 μg per ml (1, 2). Active

in mice against experimental infections caused by *B. dermatitidis* and *Cryptococcus neoformans* (1, 3).

Toxicity: LD_{50} (mice) 3 mg per kg intravenously, 17 mg per kg intraperitoneally, 12 mg per kg intramuscularly, and 46 mg per kg subcutaneously (1).

References:

1. Charney, J. *et al.* Antibiotics Ann. 228–235, 1955–1956.
2. Muller, W. H. Am. J. Botany **45:** 183–190, 1958.
3. Solotorovsky, M. *et al.* Antibiotics & Chemotherapy **8:** 364–371, 1958.
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Eumycetin

Produced by: Streptomyces sp.

Method of extraction: Washed mycelium extracted with methanol, the extract concentrated *in vacuo*, and lyophilized. Residue extracted repeatedly with ethanol, and the ethanol extract concentrated. Baryta water is added to the concentrate until no more precipitation occurs. Precipitate removed by centrifugation; excess $Ba(OH)_2$ precipitated by CO_2. An inactive precipitate forms on adding an equal volume of H_2O, and is filtered off. Eumycetin precipitated on chilling for 24 to 48 hours. Recrystallized from 50 per cent ethanol.

Chemical and physical properties: Colorless needles; m.p. 148–150°C. Soluble in methanol, ethanol, butanol, acetone, ether, chloroform, and ethyl acetate. Sparingly soluble or insoluble in water, 10 per cent HCl, and 10 per cent NaOH. Negative biuret, Fehling, ninhydrin, Millon, Molisch, Liebermann-Burchard, Sakaguchi, and Rosenheim tests. Positive $FeCl_3$ and diazo tests. No color produced by addition of concentrated sulfuric or hydrochloric acids. Ultraviolet spectrum shows peak at 302 mμ (c = 0.02 per cent in methanol).

Biological activity: Active on fungi. Inhibits after 72 hours: *Torula rubra*, *Mycoderma* sp., and *P. chrysogenum* at <0.05 μg per ml; *Oidium lactis* at 1 μg per ml; *Aspergillus oryzae* at 0.325 μg per ml; *Willia anomala* at 0.5 μg per ml; *Saccharomyces sake* at 1.0 μg per ml; *C. albicans* at 10 μg per ml; and *Trichophyton interdigitale* at 0.15 μg per ml. Active against *Nocardia* and *Streptomyces*. Not active against bacteria. *Mycobacterium* 607 inhibited by 100 μg per ml. Activity against *C. albicans* affected by horse serum. No hemolytic activity against rabbit red blood cells.

Toxicity: LD_{50} (in water with carboxymethylcellulose) 3.0 mg per kg subcutaneously in mice, 2.2 mg per kg intraperitoneally.

Reference: 1. Arai, T. and Takamizawa, Y. J. Antibiotics (Japan) **7A:** 165–168, 1954.

Eurimycin

Produced by: Streptomyces sp.

Method of extraction: Clarification of broth at pH 2.0 with 1 per cent "Kerozite" with agitation for 1 hour. Active filtrate adjusted to pH 8.2 with sodium hydroxide; the precipitate that forms is filtered off. The filtrate treated with 1.5 per cent Kerozite (2 hours of agitation). Elution with acidic methanol (0.37 per cent HCl). Neutralization of the eluate with sodium hydroxide, and concentration *in vacuo*. Addition of acetone in excess gives an inactive precipitate, and the antibiotic is precipitated as the picrate from the active layer. The solid hydrochloride may be precipitated by petroleum ether. Purification by chromatography on alumina. Inactive impurities removed with acidic methanol (10 per cent formic acid) and the antibiotic eluted with water.

Chemical and physical properties: Paper chromatography (n-butanol-propionic acid-water, 4:1:5) indicates three constituents, of which two are active and ninhydrin-positive. Light cream-colored. Sulfate very soluble in water (hygroscopic), soluble in methanol, slightly soluble in ethanol, and very slightly soluble in acetone. Practically insoluble in ether and ligroin. Precipitated as a picrate, phosphotungstate, helianthate, and reineckate. Positive ninhydrin, Sakaguchi, biuret, and creatinine tests. Creatinine test more strongly positive after acid hydrolysis. Ammonia is released during alkaline hydrolysis, with loss of a guanidino group. Negative maltol, Schiff, Fehling, and glucosamine tests. A salmon color in 3.5 per cent HCl is intensified by boiling; a pale yellow color in 10 per cent $NaHCO_3$ fades on boiling. Thermostable. Little loss in activity noted after heating for 3 hours in 5 per cent BaOH over a steam bath, or 30 minutes at 100°C at pH 2.0. The principal substance, purified by chromatography on a cellulose column and developed with n-butanol-acetic acid-water (4:1.8:5), decomposes at 155°C (chloride) and 130°C (sulfate).

Biological activity: Active on gram-positive and gram-negative bacteria and mycobacteria. Protects mice and partially protects rabbits against infection with *Staph. aureus*. Guinea pigs protected against infection with *B. anthracis*.

Toxicity: LD_0 (mice) 200 mg per kg subcutaneously; 700 mg per kg (rabbits); and 300 mg per kg (guinea pigs).

Reference: 1. Gonçalves de Lima, O. *et al.* Anais soc. biol. Pernambuco **12:** 9–17, 1954.

Eurocidin

Produced by: Streptomyces albireticuli (4), *S. eurocidicus* (2, 3), and *Streptomyces* sp. (6). *S. albireticuli* produces carbomycin, tertiomycin A, and enteromycin (10). *S. eurocidicus* also produces a eurocidin-like antibiotic (E-I), as well as azomycin and the tertiomycins. E-I may be more like "pentaene antifungal antibiotic I." A *Streptomyces* sp. (6) also produces a eurocidin-like substance (E-II) and an actinomycin.

Remarks: See also eurocidin-like antibiotic.

Method of extraction: Eurocidin: Extracted from mycelium with methanol. Crystallized from aqueous methanol (5). E-I: Broth-filtrate extracted with butanol. Extract evaporated to dryness *in vacuo*. Residue taken up in methanol and precipitated by adding ether. Small amount present in mycelium extracted with methanol (3). E-II: Methanol extracts of mycelium evaporated to dryness *in vacuo*. Residue taken up in ethyl acetate. Extract evaporated to dryness *in vacuo*. Chromatographed on alumina from an acetone solution and developed with benzene, acetone (15 per cent)-benzene, followed by acetone (30 per cent)-benzene, then acetone. Antibiotic eluted with 50 per cent methanol (6).

Chemical and physical properties: Pentaenes. *Eurocidin:* Gray-white or cream-colored substance. Does not melt up to 300°C. Insoluble or sparingly soluble in water, butanol, methanol, ethanol, ether, ethyl and butyl acetate, propylene and ethylene glycol, and acetone. Soluble in acids, alkalies, pyridine, dimethyl sulfoxide, and hydrous organic solvents (1, 5, 7–9). Ultraviolet absorption spectrum maxima at 318, 332, and 350 mμ (ethanol) (1). $[\alpha]_D^{15} = -200°$ (c = 0.25 per cent in 0.1 *N* HCl) and +22° (c = 0.25 per cent in 0.1 *N* NaOH). Positive Fehling test. Negative biuret, Molisch, and Sherivanov tests (5). Rf = 0.40 by ascending paper chromatography (methanol-butanol-water, 9:10:10) (8). C = 57.99%; H = 8.13%; N = 1.65%. No halogens or sulfur (5). *E-I:* Soluble in water, methanol, and ethanol. Very slightly soluble in acetone. Insoluble in benzene, ether, and petroleum ether. Ultraviolet absorption spectrum maxima at 318, 333, and 351 mμ (3). *E-II:* White substance. Ultraviolet absorption spectrum maxima at 310 mμ (shoulder), 322.5 mμ ($E_{1cm}^{1\%}$ 125), 338.5 mμ ($E_{1cm}^{1\%}$ 179), and 356.5 mμ ($E_{1cm}^{1\%}$ 164) (6).

Biological activity: Eurocidin: Active on yeasts, filamentous fungi, and certain protozoa, including *Trichomonas vaginalis* (1, 5). Active in mice on *T. vaginalis* (9). *E-I:* Active on yeasts and fungi but not on bacteria or *N. asteroides* (3). *E-II:* Active on *C. albicans* and *A. niger* at 25 to 50 μg per ml (6).

Toxicity: Eurocidin: LD_{50} (mice) 22 mg per kg intraperitoneally (1). *E-I:* LD_{50} (mice) 36 mg per kg (no route given) (3).

References:

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3. Utahara, R. *et al.* J. Antibiotics (Japan) **7A:** 120–124, 1954.
4. Nakazawa, K. J. Agr. Chem. Soc. Japan **29:** 647–649, 1955.
5. Nakazawa, K. J. Agr. Chem. Soc. Japan **29:** 650–652, 1955.
6. Maeda, K. *et al.* J. Antibiotics (Japan) **9A:** 125–127, 1956.
7. Shibata, M. Japanese Patent 4995, 1956.
8. Steinman, I. D. Thesis, Rutgers University, 1958.
9. Hamada, Y. Ôsaka Daigaku Igaku Zassi **7:** 237–245, 1955.
10. Miyake, A. *et al.* J. Antibiotics (Japan) **12A:** 59–64, 1959.

Eurocidin-like Antibiotic

Produced by: Streptomyces sp. belonging to the *S. reticuli* group. This organism also produces netropsin and antibiotic 2814K.

Method of extraction: Mycelium extracted with butanol.

Chemical and physical properties: Amorphous yellow pentaene. Soluble in concentrated H_2SO_4, giving a blue-violet color. Ultraviolet absorption spectrum maxima at 317, 332 to 333, and 350 mμ. Has nitrogen content and solubility properties similar to eurocidin, but does not show the $[\alpha]_D^{15} = -200°$ (0.1 *N* HCl) given for eurocidin.

Biological activity: Active on *Sacch. cerevisiae* at 1:400,000.

Reference: 1. Thrum, H. Naturwissenschaften **16:** 87, 1959.

Evericin

Produced by: Probably a *Streptomyces*.

Method of extraction: Broth acidified with oxalic acid to pH 2.0 and filtered. Broth neutralized with NaOH and adsorbed on cation exchange resin. Elution with 1.5 *N* HCl. Neutralized eluate evaporated to dryness under reduced pressure. Extraction of residue with methanol. Precipitation of

crude evericin with acetone. Further purification by chromatography on charcoal.

Chemical and physical properties: Basic antibiotic. Unstable at 100°C. Hydrochloride salt hygroscopic. Sulfate not hygroscopic. Sulfates of 370 units per mg (1 unit = 1 μg of streptomycin by diffusion assay) were obtained. Evericin diffuses more slowly than streptomycin in agar. Ultraviolet absorption spectrum shows end-absorption and a characteristic shoulder at approximately 240 mμ. Maltol test negative. Negative $FeCl_3$ and Molisch tests. Positive Tollen, biuret, and ninhydrin reactions. On chromatography of the acid hydrolysate on activated carbon, the first fractions contain four atypical amino acids; subsequent fractions contain no amino acids but guanidine. $[\alpha]_D = -20.1°$ (c = 1 per cent in water.)

Biological activity: Bacterial spectrum similar to streptomycin. Active against streptomycin-resistant strains.

Toxicity: LD_{50} (mice) 10 to 12.5 mg per kg intravenously and subcutaneously.

Reference: 1. Bodansky, M. Acta Chim. **3:** 237–241, 1953.

Exfoliatin

Produced by: Streptomyces exfoliatus (1).

Method of extraction: Broth at pH 7.0 extracted with ethyl acetate. Extract distilled *in vacuo.* Residual syrup washed with petroleum ether to precipitate exfoliatin. Crystallized from hot ethanol on cooling (1).

Chemical and physical properties: Colorless needles; m.p. 172°C. Soluble in ethanol, acetone, chloroform, and ethyl acetate. Very slightly soluble in petroleum ether, ether, and water. Positive Molisch and $FeCl_3$ tests. Negative Fehling, Tollen, and Liebermann-Burchard tests. C = 50.74%; H = 6.10%; Cl = 5.48%. $C_{27}H_{40}O_{19}Cl$ (1, 2).

Biological activity: Active on gram-positive bacteria. Less active on mycobacteria. Not active on fungi or gram-negative bacteria, except *Hemophilus* (1, 2).

Toxicity: LD_{50} (mice) 500 mg per kg subcutaneously (1, 2).

References:

1. Umezawa, H. *et al.* Japan. J. Med. Sci. & Biol. **5:** 311–316, 1952.
2. Umezawa, H. *et al.* J. Antibiotics (Japan) **5:** 466, 1952.

Fermicidin

Produced by: Streptomyces sp. similar to *S. griseolus.*

Method of extraction: Filtered broth acidified to pH 3.0 to 4.0, treated with activated carbon, eluted with 80 per cent acetone. Concentration of eluate *in vacuo* followed by extraction with chloroform. Chloroform removed under reduced pressure, and the residue adsorbed on a carbon column. Column washed with 20 per cent acetone, and fermicidin eluted with 60 per cent acetone. Acetone evaporated to dryness *in vacuo,* and the residue dissolved in benzene, absorbed on alumina, and eluted with 1 per cent methanol containing benzene. Methanol-benzene solution evaporated to dryness *in vacuo.* Recrystallization from ether.

Chemical and physical properties: Colorless needles; m.p. 96–98°C. Soluble in methanol, ethanol, chloroform, benzene, and ethyl acetate. Slightly soluble in water and ether. Insoluble in petroleum ether. Aqueous solutions are weakly acidic. $[\alpha]_D^{18} = +52.3°$ (c = 0.62 per cent in water). Stable for 1 hour at 100°C at pH 5 or less. Unstable at alkaline reaction. Ultraviolet absorption maximum at 290 mμ.

Biological activity: Active on *Sacch. formosensis* and *Sacch. pastorianus* at 0.04 μg per ml; *Sacch. cerevisiae* at 0.1 μg per ml; *Hansenula anomala* and *Torula rubra* at 0.2 μg per ml; *Candida krusei* at 0.5 μg per ml; and *Trichomonas vaginalis* at 0.2 μg per ml. Active on influenza virus; not active against bacteria and filamentous fungi.

Toxicity: LD_{50} (mice) 180 mg per kg intravenously; and (rats) 2 mg per kg.

References:

1. Igarasi, S. J. Antibiotics (Japan) **7B:** 221–225, 1954.
2. Wada, S. *et al.* Chem. Abstr. **52:** 16703f, 1958.

Fervenulin

Produced by: Streptomyces fervens.

Method of extraction: Filtration of the broth at pH 8.0. Filtrate extracted with ¼ volume of methyl chloride at pH 6.0. Concentration to an oil. Extraction of the oil with the upper phase of the solvent system acetone-n-hexane-water (5:3:1). Concentration of the solvent yields crystalline fervenulin. Pure fervenulin obtained by fractional crystallization from the solvent system ethyl acetate-acetone (3:1) or by countercurrent distribution in the solvent system benzene-methanol-water (1:1:0.2).

Chemical and physical properties: Brilliant yellow orthorhombic crystals; m.p. 178–179°C (decomposition). Sublimes 70°C at 10 μ pressure. C = 43.83%; H = 3.73%; N = 35.99%; O = 17.27%. Molecular weight (radiological), 189; vapor pressure, 190; saponification, 186. Molecular weight,

193.17, with suggested empirical formula $C_7H_7 \cdot N_3O_2$. Infrared absorption spectrum given in reference 1. Light absorption maxima at 239, 270 to 280, and 340 mμ in pH 7.85 phosphate buffer. At pH 10 the maxima at 239 and 340 mμ are destroyed but the maximum at 270 to 280 mμ remains unchanged. Neutral compound, soluble in practically all common organic solvents except hydrocarbons. Soluble in water (2 mg per ml cold; 40 mg per ml hot). Destroyed by alkalies. Not affected by 6 *N* HCl at 100°C for 40 hours. Positive copper sulfide test. Negative ninhydrin, $FeCl_3$, Tollen, Benedict iodoform, biuret, Hinsberg, and Sakaguchi tests (1).

Biological activity: In vitro: Low degree of activity against gram-positive and gram-negative bacteria. Of 23 strains of bacteria tested, only three were sensitive to less than 25 μg per ml. Low degree of activity against some fungi (*Histoplasma capsulatum*) and some protozoa (2). *In vivo:* Limited activity against trichomonas infections in mice and hamsters. No activity in mice against experimental infections caused by *Streptococcus hemolyticus*, *K. pneumoniae*, *Histoplasma capsulatum*, and *Cryptococcus neoformans* (2). Inactive against sarcoma 180, Ehrlich carcinoma ascites, Walker adenocarcinoma, Murphy-Sturm lymphosarcoma, Jensen sarcoma, and Guerin adenocarcinoma in mice or rats (2).

Toxicity: LD_{50} (mice) 65 mg per kg intraperitoneally; (hamsters) 11.2 mg per kg (2).

References:

1. Eble, T. E. *et al.* Antibiotics Ann. 227–229, 1959–1960.
2. DeBoer, C. *et al.* Antibiotics Ann. 220–226, 1959–1960.

Filipin

Produced by: Streptomyces filipinensis (2, 8). This culture also produces an unrelated antifungal substance, which is chloroform-soluble (8).

Method of extraction: I. Ethyl acetate, ether, or n-butanol extracts of filtered broth evaporated to 1/10 volume, hexane or Skellysolve B added, and resulting precipitate washed with petroleum ether and dried *in vacuo*. Extracted from mycelium with methanol and butanol. Crystallized by trituration with chloroform and recrystallized from methanol (2, 8). II. Broth mixed with diatomaceous earth, filtered, and broth extracted with ethyl acetate. Solvent extract concentrated *in vacuo*, and filipin precipitated with petroleum ether. Crystallized from chloroform (3).

Chemical and physical properties: Yellow, neutral conjugated pentaene. Fine needles. At 147°C undergoes transition to second, partially degraded form with m.p. 195–205°C (decomposition). Very soluble in dimethylformamide and pyridine. Soluble in methanol, ethanol, n-butanol, isopropyl alcohol, t-butyl alcohol, ether, ethyl acetate, amyl acetate, and glacial acetic acid. Nearly insoluble in water, chloroform, 50 per cent ethanol, methylene chloride, and Skellysolve B. λ_{max}^{MeOH} 322 ($E_{1cm}^{1\%}$ 910), 338 ($E_{1cm}^{1\%}$ 1360), and 355 ($E_{1cm}^{1\%}$ 1330) with a shoulder at 305. No shift in acid or alkali. Infrared spectrum given in reference 3. $[\alpha]_D^{22} = -148.3°$ (c = 0.89 per cent in methanol). Positive Molisch test. Negative ninhydrin, biuret, Benedict, Tollen, 2,4-dinitrophenylhydrazine, and $FeCl_3$ tests. Deep blue color in concentrated HCl or H_2SO_4, but not in HNO_3. Thermolabile. Photosensitive in O_2. Subject to auto-oxidation. Stable in dark in air at 5°C. Seventy per cent inactivated by 10 per cent horse serum in a methanol solution. Rf values on paper chromatography given in references 2 and 3. Tentative empirical formula: $C_{32}H_{54}O_{10}$ (11). Saponification equivalent, 574. Filipin undergoes a nonreversible, autocatalytic degradation in concentrated methanolic or ethanolic solutions standing at 4°C, to a white, biologically and optically inactive, crystalline polyene, $C_{30}H_{50}O_{10}$, m.p. 195–205°C, with an infrared spectrum nearly identical to filipin and λ_{max}^{MeOH} at 318 ($E_{1cm}^{1\%}$ 1050), 303 ($E_{1cm}^{1\%}$ 1170), 290 ($E_{1cm}^{1\%}$ 770) and a shoulder at 281. Gives a wine-red color in concentrated H_2SO_4. Basic hydrolysis of filipin yields a biologically inactive acid thought to be derived from a lactone ring (2, 3, 10).

Biological activity: Active on fungi and yeasts; slightly active on *Trichomonas foetus*. Not active on bacteria. Naturally occurring fungal rot of pea and tomato seeds (caused by penicillia, aspergilli, and phycomycetes for the most part) almost completely suppressed by soaking seeds for 3 hours in 25 to 100 μg per ml of filipin. Partial protection of tomato plants from gray leaf spot (*Stemphylium solani*) in greenhouse tests (1, 2). Active on the damping-off stage of safflower root rot (*Pythium* sp.) (5), downy mildew of cucumber (*Pseudoperonospora cubensis*) (6) and broccoli (*Peronospora parasitica*) (7), black mold of onion, corn leaf blight, collar rot of tomatoes, bitter rot of apples, and tomato wilt (8). Improves emergence of cucumber, vegetable marrow, and muskmelon (4). Activity on *Penicillium oxalicum* reversed by cholesterol (10). Intermediate activity on sarcoma 180 (mice) (8).

Toxicity: LD_{50} (mice) 17 mg per kg intraperitoneally (8). No effect on pea and tomato seed germination at 100 μg per ml applied by soaking.

No toxic effect on young tomato plants sprayed with 415 μg of filipin per ml of 25 per cent methanol (1, 2).

References:

1. Gottlieb, D. *et al.* Plant Disease Reptr. **39:** 219, 1955.
2. Ammann, A. *et al.* Phytopathology **45:** 559–563, 1955.
3. Whitfield, G. B. *et al.* J. Am. Chem. Soc. **77:** 4799–4801, 1955.
4. Wallen, V. R. and Bell, W. Plant Disease Reptr. **40:** 129–132, 1956.
5. Gattani, M. L. Plant Disease Reptr. **41:** 160–164, 1957.
6. Ark, P. A. and Thompson, J. B. Plant Disease Reptr. **41:** 452–454, 1957.
7. Natti, J. J. Plant Disease Reptr. **41:** 780–788, 1957.
8. British Patent 783,486, September 25, 1957.
9. Field, J. B. *et al.* Cancer Research **18:** (Suppl. 1) 492, 1958.
10. Sloneker, J. H. Thesis, University of Illinois, 1958.
11. Tingstad, J. E. and Garrett, E. R. J. Am. Pharm. Assoc., Sci. Ed. **49:** 352–355, 1960.

Flavacid

Produced by: Streptomyces sp. closely related to *S. flavus*.

Method of extraction: The mycelium of the *Streptomyces* washed with water, dried, and crushed into powder. Extraction of mycelium with 1:1 mixture of butyl acetate-acetone, or with ethyl acetate. These solvents removed from the mycelium a toxic antibiotic (D-substance), which is discarded. Mycelium extracted with methanol and discarded. Methanol evaporated *in vacuo*, and the solid residue extracted with butanol at pH 5.0. Butanol-extract washed with acidic water (pH 2.0) and adjusted to pH 7.0. After concentration of butanol to a small volume, flavacid is precipitated out with saturated alkaline methanol. Yield: 500 to 800 mg per 100 gm of dry mycelium.

Chemical and physical properties: Hexaene (2): Weak acid, active and unstable under acidic conditions. *Sodium salt:* soluble in methanol and aqueous acetone and moderately soluble in water and ethanol; slightly soluble in acetone and butanol; insoluble in ether, ethyl acetate, chloroform, and benzene. Indicator properties: green in acid solutions, yellow at neutrality, red at alkaline pH. Inactive at neutrality and at alkaline reaction. Ninhydrin and $FeCl_3$ reactions negative. Ultraviolet absorption maxima at 335, 355, and 373 mμ (methanol); m.p. 102–105°C (1). A tetraene (peaks at 293, 306, and 324 mμ (ethanol)) is also present in small quantities, as with endomycin. The hexaene from flavacid was shown to differ from that of endomycin. Not enough of the tetraene was present for comparison with the endomycin tetraene (2).

Biological activity: Active *in vivo* against gram-positive bacteria (3 to 6 μg per ml); very slight activity against gram-negative bacteria. Active against yeasts and filamentous fungi, including *Trichophyton* and *C. albicans*. Inactivated by human serum. Experimental systemic infections of mice and guinea pigs with *C. albicans* do not respond to flavacid treatment. Oral administration of flavacid reduces the number of *C. albicans* in the digestive tract.

Toxicity: LD_{50} (mice) 50 mg per kg intraperitoneally.

Utilization: Some effect in the treatment of trichophytosis in man, when applied topically. Excellent results obtained in the topical treatment of *Trichomonas vaginalis* in human beings.

References:

1. Takahashi, J. J. Antibiotics (Japan) **6A:** 117–121, 1953.
2. Vining, L. C. and Taber, W. A. Can. J. Chem. **37:** 1461–1466, 1957.

Flavensomycin

Produced by: Streptomyces sp. resembling *S. tanashiensis* (2).

Synonym: Antibiotic 829 (1).

Method of extraction: Broth-filtrate extracted with benzene at pH 7.5 to 8.0. Extract concentrated *in vacuo* until precipitate appears. Precipitate washed with petroleum ether and extracted with acetone. Acetone concentrated and petroleum ether added to precipitate crude flavensomycin. Purification by chromatography on alumina from a benzene solution, washing with ethyl acetate, ethanol-ethyl acetate, and elution with methanol (2, 3).

Chemical and physical properties: Pale yellow, odorless tabular crystals; m.p. 152° ± 2°C. Soluble in water, lower alcohols and acetates, benzene, chloroform, pyridine, acetone, dioxane, and propylene glycol. Insoluble in ether, petroleum ether, hexane, carbon tetrachloride, and carbon disulfide. Contains N, but no S or halogen. Ultraviolet absorption spectrum maximum at 251 mμ (methanol). Infrared data given in reference 2. Stable, in dry form, in organic solvents and in neutral aqueous solutions at <12°C. Positive Molisch, Fehling, and Ehrlich (diazo) tests. Negative Tol-

len, Seliwanoff, Millon, Liebermann, Sakaguchi, biuret, and ninhydrin tests (1–3).

Biological activity: Most active on *Saccharomyces* and *Penicillium* (0.5 μg per ml). Less active (5 to 50 μg per ml) on other fungi. No activity on bacteria. Active on insects (*Musca domestica* and *Locusta migratoria*) (2). Strongly inhibits mitosis at pre-prophase in *Allium cepa* root cells (1).

Toxicity: LD_{50} (mice) 1 mg per kg intraperitoneally, 2 mg per kg subcutaneously, and 25 mg per kg orally (1, 2).

References:

1. Craveri, R. and Veronesi, U. Riv. biol. (Perugia) **49:** 89–97, 1957.
2. Craveri R. and Giolitti, G. Nature, London **179:** 1307, 1957.
3. Craveri, R. *et al.* Nuovi ann. igiene e microbiol. **9:** 185–187, 1958.

Flaveolin

Produced by: Streptomyces sp. resembling *S. flaveolus*.

Method of extraction: Culture medium adjusted to pH 2.0 and filtered. Filtrate decolorized with charcoal at pH 2.0, then antibiotic adsorbed on charcoal at pH 7.0. Elution with 80 per cent acetone (pH 1). Acetone evaporated off in flash-evaporator and Ca^{++} removed as oxalate. Filtrate extracted with butanol at pH 8.0. Re-extraction into water (pH 1.0). Separated from impurities by countercurrent distribution (butanol and 0.067 *M* Sorensen's buffer at pH 5.5). Active fractions transferred from acidic water into chloroform repeatedly. Precipitated from chloroform on addition of ether.

Chemical and physical properties: Basic substance. Possible quinonoid structure. *HCl salt:* Yellowish powder. Soluble in water, methanol, ethanol, and propanol. Scarcely soluble in acetone, benzene, butanol, and chloroform. Insoluble in ether, petroleum ether, and ethyl acetate. Lemon-yellow in acidic solution, brown at neutrality, and reddish at alkaline pH. Most stable at acid pH. Decolorized by H_2O_2 in presence of Na_2CO_3. Positive Liebermann nitroso reaction and Pauly tests. Negative $FeCl_3$, Millon, biuret, Sakaguchi, ninhydrin, and Fehling (black color, no precipitate) tests. *Picrate:* m.p. 126–128°C (decomposition). *Reineckate:* m.p. 147–150°C (decomposition). *Base:* Soluble in butanol, chloroform, and isoamyl alcohol. Not precipitated by methyl orange or flavianic acid. Rf = 0.74 (wet butanol) or 0.83 (3 per cent NH_4Cl) by paper chromatography.

Biological activity: Active on gram-positive and gram-negative bacteria, mycobacteria, yeasts, and fungi.

Toxicity: Mice tolerate 10 mg per kg intravenously, but 50 mg per kg is lethal.

Reference: 1. Takahashi, B. J. Antibiotics (Japan) **6:** 11–20, 1953.

Flavocidin

Produced by: Streptomyces sp.

Synonym: Possibly related to antibiotic F 256.

Method of extraction: Activity present primarily in the mycelium. Mycelium exhaustively extracted with boiling methanol. Methanol concentrated, and antibiotic precipitated on addition of water. Recrystallized from boiling chloroform.

Chemical and physical properties: Colorless needles; m.p. 144–145°C. Very soluble in methanol, ethanol, acetone, ethyl acetate, and chloroform. Soluble in benzene and ether; sparingly soluble in water; insoluble in petroleum ether and ligroin. Ultraviolet absorption spectrum maximum at 275 mμ ($E^{1\%}_{1cm}$ 2.01) in methanol. Infrared spectrum given in reference 1. $[\alpha]_D^{20} = +94°$ (c = 1 per cent in methanol). Positive bromine-chloroform, nitroprusside, Molisch, diazo, and m-dinitrobenzene tests. Negative ninhydrin test. Addition of vanillin to a glacial acetic acid solution gives a red color, indicating a phenolic hydroxyl group. $C_{34}H_{55-59}NO_9$: C = 65.20%; H = 9.12%; N = 2.05%. Molecular weight, 625 to 643.

Biological activity: Active on *Micrococcus flavus* at 0.003 μg per ml and on *Sarcina lutea*. Not active on other bacteria. Active on influenza virus *in vitro*.

Toxicity: LD_{50} (mice) 15.6 to 31.25 mg per kg intraperitoneally.

Reference: 1. Shibata, M. *et al.* Ann. Rept. Takeda Research Lab. **17:** 16–18, 1958.

Flavofungin

Produced by: Streptomyces flavofungini (2). (This culture also produces desertomycin.) *Streptomyces* sp. (These cultures also produce an antibacterial antibiotic (4).)

Synonym: Antifungal antibiotic SA 1X (1); said to be related to mycoticin (4).

Method of extraction: Present in mycelium and broth (2).

Chemical and physical properties: Fine yellow to yellow-green needles (2, 4). Soluble with difficulty in water. Yellow fluorescence in ultraviolet light. Ultraviolet absorption spectrum maxima at 263 and 368 mμ (3). Forms a stable acetyl derivative (4). Slow diffusibility in agar. Reversibly bound by serum proteins (2).

Biological activity: Active on yeasts, filamentous fungi, and *N. asteroides*. Not active on bacteria, except *B. subtilis* and *Staph. aureus* at >100 μg

per ml. Active on *C. albicans* in the mouse intestinal tract (2). Not active on streptomycetes (4).

Toxicity: LD_{50} (mice) 25 mg per kg intraperitoneally (2); also 40 to 50 mg per kg intraperitoneally, 2.5 to 4.0 mg per kg intravenously, and 750 to 1200 mg per kg orally (3). Toxic to conjunctiva (3).

Utilization: Used topically, orally, and parenterally. Active on vaginal trichomoniasis (3).

References:

1. Uri, J. Acta Physiol. Acad. Sci. Hung. **11:** (suppl.) 103–104, 1957.
2. Békési, I. Nature, London **181:** 908, 1958.
3. Kelentey, B. *et al.* Abstr. Communs. Symposium on Antibiotics, Prague, pp. 67–68, 1959.
4. Uri, J. Arzneimittel-Forsch. **9:** 175–181, 1959.

Fradicin

Produced by: Streptomyces fradiae (1).

Synonym: Factor X (1).

Method of extraction: I. Broth-filtrate extracted with n-butanol at pH 7.0. Extract concentrated to dryness *in vacuo*. Residue taken up in 95 per cent ethanol, concentrated, and added dropwise to acetone. Solution concentrated *in vacuo* and added dropwise to petroleum ether. Supernatant discarded. Residual oil dissolved in t-butanol and lyophilized (1). II. Culture filtered with the aid of Super-Cel. Cake extracted with isopropanol-ethyl acetate (1:10). Extract (A) concentrated to give either a precipitate or a tarry residue. Ethanol solution of such residues chromatographed on Super Filtrol Celite, developed with ethanol, then with 10 per cent (volume per volume) ammonia in ethanol (3). Active fractions concentrated and precipitated by scratching the side of the flask containing the concentrate. Extract A can also be treated by adjusting the pH to 5.8 and stirring with Stoddard solvent to separate two phases. Lower phase adjusted to pH 8.0 to 8.5 to precipitate fradicin. Crystallized from hot ethylene dichloride on cooling. Further purified by chromatography on Florisil with 1,4-dioxane as solvent and developer (3).

Chemical and physical properties: Weak base. Light green-yellow crystals. No definite melting point. Darkens at about 180–300°C. Most soluble in dioxane, propylene glycol, ethylene dichloride, and other chlorinated hydrocarbon solvents. Insoluble or very sparingly soluble in cyclohexane, xylene, and petroleum ether. Slightly soluble in water. Ultraviolet absorption spectrum maxima at about 242 and 292 mμ, with a shoulder at 265 mμ (absolute methanol) (1). Ultraviolet spectrum of this preparation shows the presence of a contaminating hexaene, probably the same as the biologically inactive hexaene later reported to be produced by this strain of *S. fradiae* (8). $[\alpha]_D^{25}$ = +65° (c = 1.0 per cent in 1,4-dioxane). More stable at neutrality than at pH 2.0. Neutral equivalent, 498 to 514. $C_{30}H_{34}N_4O_4$: C = 70.29%; H = 6.59%; N = 10.86%; O—CH_3 = 11.95%. Molecular weight, about 500 (Barger). *Hydrochloride:* Needles. Darken on exposure to light. Alkali fusion yields a volatile product which gives positive pine-splint and Ehrlich (pyrrol) tests (1–3).

Biological activity: Active on yeasts and fungi. Active on certain protozoa, such as *Trypanosoma cruzi* and *Endamoeba histolytica*. Not active on bacteria (1, 5, 7). Activity strong at pH >7.0; greatly reduced at lower pH (1, 2). Cysteine and other reducing agents decrease antifungal activity (4), as do oleate and Tween 80 (6).

Toxicity: LD_{50} (mice) 4 mg per kg intraperitoneally and orally. Irritating to rabbit skin at 50 μg per gm of a hydrophilic ointment (5).

References:

1. Swart, E. A. *et al.* Proc. Soc. Exptl. Biol. Med. **73:** 376–378, 1950.
2. Hickey, R. J. and Hidy, P. H. Science **113:** 361–362, 1951.
3. Hidy, P. H. and Hickey, R. J. Arch. Biochem. Biophys. **34:** 67–71, 1951.
4. Waksman, S. A. *et al.* Bull. World Health Organization **6:** 163–173, 1952.
5. Schwartz, J. A. *et al.* Trans. 11th Veterans Admin. Conf. Tuberc. 86–93, 1952.
6. Hickey, R. J. Arch. Biochem. Biophys. **46:** 331–336, 1953.
7. Packchanian, A. Am. J. Trop. Med. Hyg. **2:** 243, 1953.
8. Steinman, I. D. Thesis, Rutgers University, 1958.

Fradicin-like Antibiotic

Produced by: Streptomyces sp. differing from *S. fradiae* and *S. roseoflavus* (mycelin-producer).

Remarks: Authors (1) believe that the antibiotic may also resemble mycelin. May be contaminated with, or actually be a hexaene.

Method of extraction: Mycelium extracted with methanol. Extract washed with water and concentrated *in vacuo*. Ether added to the concentrate to give a precipitate.

Chemical and physical properties: Dark yellow powder. Soluble in butanol, methanol, ethanol, and pyridine. Slightly soluble in water. Insoluble in chloroform, benzene, acetone, ethyl acetate, ether, and petroleum ether. Ultraviolet absorption spectrum maxima at 243, 294, 335, 355, and 373 mμ.

Biological activity: Active on *Sacch. sake* and *C. albicans*.

Toxicity: LD_{50} (mice) 15 mg per kg intraperitoneally.

Reference: 1. Utahara, R. *et al.* J. Antibiotics (Japan) **12A:** 73–74, 1959.

Framycetin

Produced by: Streptomyces lavendulae (1, 2, 8). This strain was later identified as *S. fradiae* (13). *Streptomyces* sp. (6).

Synonyms: Soframycin, antibiotic E. F. 185 (6, 8), neomycin (14).

Method of extraction: I. Broth-filtrate treated with sodium hexametaphosphate, pH adjusted to 8.5, and adsorption on IRC-50. Eluted with aqueous 5 per cent triethylamine or dilute HCl (pH 2.0). Adsorbed on triethylamine-washed IRC-50 under different pH conditions and eluted with aqueous acidic methanol; pH of eluate adjusted to 5 with triethylamine, and framycetin precipitated with 40 per cent triethylamine sulfate in absolute methanol. Purification by chromatography on alumina. Crystallized as the picrate (1, 6, 8). II. Broth-filtrate adsorbed on carbon at pH 8.2. Carbon washed, then eluted with methanol-HCl (10:1), followed by methanol. Added to ethyl ether to precipitate framycetin (1, 8). III. Whole culture adjusted to pH 3 or heated to 35–50°C, and filtered. Adsorbed on carbon or silica gel at pH 7, and eluted with acidic alcohol. Eluates purified by treatment with carbon, and with IR-100 at pH 4.0, followed by adsorption on IRC-50. Eluted with acidic alcohol (pH 2.0). Further purified by double decomposition between sodium *p*-(*p'*-hydroxyphenylazo)benzene sulfonate and a crude salt of the antibiotic, and other salt interconversions (8).

Chemical and physical properties: Complex, containing three components (1). *Base:* m.p. about 195°C (decomposition). No characteristic absorption in ultraviolet light. $[\alpha]_D^{18} = +64°$ (c = 1 per cent in water). Stable to autoclaving for 30 minutes at pH 8.4 and for 8 hours at 25°C at pH 2 to 10. C = 46.6%; H = 7.3%; N = 12.8%; O = 33.1%; Van Slyke N = 11.35% (as nitrogen); acetyl index = 17.7%. $C_{23}H_{44}N_6O_{13}$. All hydroxyl groups are reported to be in the form of secondary alcohols. Rf = 0.22 (water-saturated n-butanol-2 per cent *p*-toluenesulfonic acid, Whatman No. 4 paper, 15 hours at 24°C (1, 8)). *Hydrochloride:* Amorphous white powder. Soluble in water and aqueous methanol. Insoluble in acetone, ether, and other common organic solvents. $[\alpha]_D = +57°$ (c = 1 per cent in water). Green color with the anthrone test. Negative Elson-Morgan and Sakaguchi tests. All N present is in the form of primary amino groups. Acid hydrolysis or methanolysis products include neamine, a diamino hexose ($C_6H_{12}O_3N_2$ or $C_6H_{14}O_4N_2$), and a pentose. The amino sugar gives positive ninhydrin, Elson-Morgan, and ammoniacal $AgNO_3$ tests, but a negative Keller-Kiliani (tor desoxy-2-hexoses) test. Rf = 0.17 (n-butanol-acetic acid-water, 50:25:25). The dipicrate of this amino sugar has a m.p. of 126°C. Pentose gives positive Tollen and Seliwanoff tests, reduces benzidene and tetrazolium, and gives a negative Keller-Kiliani test (3, 6, 10). *Picrate:* m.p. 189°C (decomposition, corrected). $[\alpha]_D^{18} = -34°$ (c = 0.5 per cent in methanol (1). *Sulfate:* Soluble in water. Insoluble in methanol, acetone, and ethyl ether. $[\alpha]_D = +44°$ (c = 1 per cent in water) (8). *p-(p'-Hydroxyphenylazo)benzene sulfonate:* Crystals; m.p. 250°C. Soluble in methanol. Scarcely soluble in ethanol. Insoluble in water, butanol, acetone, ether, and benzene (8).

Biological activity: Active on gram-positive and gram-negative bacteria, including mycobacteria, *Proteus*, and *Pseudomonas* spp. No cross-resistance with streptomycin (5, 9, 11). Active in mice on *Staph. aureus, E. coli, Sal. typhosa, Sal. typhimurium, Sal. paratyphi* (A and B), *D. pneumoniae, M. tuberculosis* var. *hominis* H37Rv, and *B. anthracis* (8).

Toxicity: LD_{50} (mice) 40 to 50 mg per kg intravenously, 250 to 275 mg per kg intraperitoneally, 450 mg per kg subcutaneously, and >5 gm per kg orally. LD_{50} (rat) 450 mg per kg intramuscularly, 700 mg per kg subcutaneously (8). Toxic to the eighth cranial nerve (7). Chronic toxicity tests showed that isonicotinic acid administered to guinea pigs with framycetin overcomes the toxicity of the antibiotic at certain levels (4).

Utilization: Bowel sterilization (12). Pulmonary disease (7).

References:

1. Hagemann, G. T. Thesis, University of Paris, 1952.
2. Decaris, L. J. Ann. pharm. franc. **11:** 44–46, 1953.
3. Penáu, H. *et al.* Ann. pharm. franc. **11:** 431–438, 1953.
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5. Lutz, A. *et al.* Strasbourg med. **4:** 431–433, 1953.
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7. Sors, C. and Trocme, Y. Presse méd. **61:** 364–365, 1954.
8. Penáu, H. *et al.* French Patent 1,051,202, January 14, 1954.
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10. Saito, A. and Schaffner, C. P. Resumés 3rd Intern. Congr. Biochem. 98, 1955.
11. Lutz, A. and Hofferer, M. J. Rev. immunol. **19:** 68–85, 1955.
12. Shidlovsky, B. A. *et al.* Antibiotics Ann. 118–121, 1955–1956.
13. Waksman, S. A. Neomycin. The Williams & Wilkins Co., Baltimore, 1958.
14. Rinehart, K. L., Jr. *et al.* J. Am. Chem. Soc. **82:** 3938–3946, 1960.

Fungichromin

Produced by: Streptomyces cellulosae (1). This strain also produces an actinomycin.

Synonym: Fungichromatin, a closely related compound (1).

Method of extraction: Extracted from broth-filtrate with ethyl or amyl acetate, or other organic solvents, and from mycelium with methanol, ethanol, or acetone. Extracts concentrated *in vacuo* to precipitate a fungichromin-actinomycin mixture. Separated by repeated crystallization from methanol.

Chemical and physical properties: Conjugated pentaene (3). Pale yellow crystals; m.p. 205–210°C (uncorrected). Soluble in methanol, ethanol, butanol, acetone, pyridine, and dimethylformamide. Insoluble in water and aliphatic hydrocarbons. Ultraviolet absorption spectrum maxima at 322.5, 338.5, and 356.5 mμ, with a shoulder at 310 mμ (in organic solvents). Infrared data given in reference 1. Violet color, changing to blue in concentrated sulfuric acid. Positive Tollen (slow), $KMnO_4$, and bromine tests. Hydrogenation product is a white, waxy solid, with no antifungal activity. Mild acetylating conditions destroy antibiotic activity but not characteristic ultraviolet absorption. C = 60.93%; H = 8.65%; O = 30.42%. Molecular weight, 688.8. $C_{35}H_{60}O_{13}$. Contains 10 to 11 hydroxyl groups, and 3 to 4 C—CH_3 groups, but no methoxyl or acetoxyl groups, N, S, or halogens. Sodium periodate oxidation yields, among other products, 2-methyl-2,4,6,8,10-dodecapentaenedial (1, 3).

Biological activity: Active on yeasts and fungi: *C. albicans*, 6.25 to 12.5 μg per ml; *Blastomyces dermatitidis*, 0.78 μg per ml; *A. niger*, 3 to 25 μg per ml; *Trichophyton mentagrophytes*, 12.4 to 50 μg per ml; *Fusarium oxysporum*, 10 μg per ml. Active on peach brown rot (*Sclerotinia fructicola*) (1, 2).

Toxicity: LD_{50} (mice) 16.4 mg per kg intraperitoneally. Mice tolerate 1000 mg per kg orally (1).

References:

1. Tytell, A. A. *et al.* Antibiotics Ann. 716–718, 1954–1955.
2. Szkolnik, M. and Hamilton, J. M. Plant Disease Reptr. **41:** 289–292, 1957.
3. Cope, A. C. and Johnson, H. E. J. Am. Chem. Soc. **80:** 1504–1506, 1958.

Fuscomycin

Produced by: Streptomyces fuscus.

Synonyms: Related to flaveolin. Differentiated from luteomycin and xanthomycin (1).

Method of extraction: Adsorbed from broth on carbon or cation exchange resin. Eluted with acidified acetone, methanol, or ethanol.

Chemical and physical properties: Basic substance. *Hydrochloride:* Decomposes at 180°C. Soluble in methanol, ethanol, butanol, and acetone. Insoluble in ether, ethyl acetate, butyl acetate, benzene, petroleum ether, chloroform, and carbon tetrachloride. Negative Sakaguchi, Molisch, Benedict, glucosamine, maltol, Fehling, $FeCl_3$, and ninhydrin tests. End-absorption of ultraviolet light.

Biological activity: Active on gram-positive bacteria; less active on gram-negative bacteria. Cross-resistance with streptomycin and streptothricin.

Toxicity: Mice tolerate injections (no route given) of 500 mg per kg.

References:

1. Ishida, N. and Miyazaki, J. J. Antibiotics (Japan) **5:** 481–487, 1952.
2. Hata, T. Japanese Patent 5046, 1953.

Gancidins

Produced by: Streptomyces sp.

Method of extraction: Filtered broth treated with activated carbon. Elution with 50 per cent acetone (pH 2.0). Eluate adjusted to pH 6.0, filtered, concentrated *in vacuo*, and filtered again. Evaporated to dryness *in vacuo* and residue taken up in a small volume of methanol. Addition of ether precipitates crude gancidin. The antibiotic could also be extracted with organic solvents at acid pH and re-extracted into water at alkaline pH. Purified and fractionated by countercurrent distribution (chloroform-McIlvaine's buffer). With the buffer at pH 4.25, three biologically active fractions are obtained, I, II, and III. Fraction II is concentrated and redistributed countercurrently in the above system. The major active fraction is taken to dryness. An ether solution of the residue is concentrated to give gancidin A crystals. Fraction I is redistributed countercurrently in the above system with the buffer at pH 2.5. The fraction in the first four tubes is concentrated and ether added to give gancidin W. Gancidin W recrystallized from chloroform. Fraction III contains two components (1, 2).

Chemical and physical properties: Gancidin A: Basic substance. Orange columnar crystals. Changes to dark orange at 110°C, then to black at 290°C. Soluble in chloroform, methanol, ethanol, acetone, and water. Slightly soluble in ethyl acetate, benzene, carbon tetrachloride, and ether. Insoluble in petroleum ether. Ultraviolet absorption spectrum maxima at 206 mμ ($E^{1\%}_{1cm}$ 324), 265 mμ ($E^{1\%}_{1cm}$ 248), and 340 mμ ($E^{1\%}_{1cm}$ 28) in 0.1 *N* HCl. Infrared absorption spectrum data given in reference 1. Positive Fehling test. Negative ninhydrin, 2,4-dinitrophenylhydrazine, and $FeCl_3$ tests. More stable to heat at acid than at alkaline pH. $C_{43}H_{58-60}N_6O_{14}$: C = 58.7%; H = 6.68%; N = 9.49%. Molecular weight, 1002 (Rast) or 883. *Hydrochloride:* Needles. *Gancidin W:* Neutral substance. White platelets; m.p. 163–164°C. Soluble in methanol, ethanol, and acetone. Slightly soluble in ethyl acetate, carbon tetrachloride, ether, and water. Insoluble in petroleum ether. Ultraviolet absorption spectrum maximum at 206 mμ ($E^{1\%}_{1cm}$ 296). Infrared data given in reference 1. Negative biuret, ninhydrin, Ehrlich, Fehling, Benedict, Tollen, Molisch, 2,4-dinitrophenylhydrazine, ferrous hydroxide, $FeCl_3$, and Br_2 tests. $C_{11}H_{17-19}O_2N_2$: C = 63.04%; H = 8.7%; N = 13.65%. Molecular weight, 210 (2).

Biological activity: Later work did not confirm early reports of good antitumor activity *in vivo*. Gancidin A is said to resemble xanthomycin A in certain respects (1, 2). *Gancidin A:* Very active on gram-positive bacteria. Active on Ehrlich ascites carcinoma *in vitro* but not *in vivo*. *Gancidin W:* No activity on *B. subtilis*. Very slight activity at >5 mg per kg against Ehrlich ascites carcinoma in mice (2).

Toxicity: Gancidin A: LD_{50} (mice) 80 μg per kg intravenously. *Gancidin W:* LD_{50} (mice) 80 mg per kg intravenously (2).

References:

1. Aiso, K. *et al.* J. Antibiotics (Japan) **9A:** 97–101, 1956.
2. Wakaki, S. *et al.* J. Antibiotics (Japan) **11A:** 150–155, 1958.

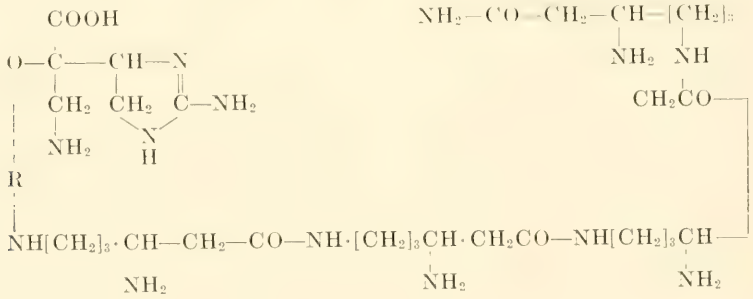
Geomycin

Produced by: Strains of *Streptomyces xanthophaeus* (1, 3).

Synonym: Streptothricin-like antibiotic.

Method of extraction: Broth-filtrate chromatographed on IRC-50 (buffered with 0.2 *M* Na_3PO_4 buffer at pH 6.5) and eluted with 0.5 *N* HCl. Active fractions adjusted to pH 6.0 with IR-4B, then concentrated to dryness *in vacuo*. Adsorbed on active carbon and eluted with weak H_2SO_4 (pH 2 to 3). Sulfate ions precipitated as Ba salt, and antibiotic converted to an oily picrate. Purification by salt conversion (hydrochloride → heliantathate → hydrochloride → base). Dried and powdered mycelium extracted with 1 *N* HCl-methanol (1:1), then purified by adsorption on IRC-50.

Chemical and physical properties: Complex: Contains four to six closely related components, varying with the producing strain. Faintly yellow powder. Ultraviolet absorption spectrum shows end-absorption only (water). Infrared spectrum given in reference 3. Positive ninhydrin and Elson-Morgan tests. Weakly positive Sakaguchi test and tests for carbohydrates and sugars. Light blue color with biuret test. Negative maltol, $FeCl_3$, and 2,4-dinitrophenylhydrazine reactions. $(C_6H_{12}O_2N_2)_{8-10}$: C = 49.48%; H = 8.91%; N = 20 to 25%. Acid hydrolysis products include NH_3, CO_2, L-β-lysine, and an amino acid, geamine, which is isomeric with or identical to streptolidine (roseonine) from streptothricin, streptolin, and roseothricin (see streptothricin-like antibiotic). *Geamine:* $C_6H_{12}O_3N_4$. *Geamine dihydrochloride:* Colorless needles; m.p. 208–213°C. $[\alpha]_D^{18}$ = +57.5° (c = 1.9 per cent in H_2O). Geomycin may contain a peptide moiety made up of glutamic acid, aspartic acid, serine, threonine, glycine, and alanine (2–4). It contains at least four β-lysine residues connected at the ε-amino groups. Geamine is bound by its hydroxyl to a sugar or amino sugar moiety (R). Partial structure (5):



Helianthate: Red platelets (from dilute methanol); m.p. 205–215°C (decomposition). *Hydrochloride:* Hygroscopic, colorless powder. Soluble in water and methanol. Less soluble in ethanol. Insoluble in acetone, ethyl acetate, ether, and petroleum ether. $[\alpha]_D^{20} = +16.0° \pm 0.4°$ (c = 3.25 per cent in water). Molecular weight, 1650 ± 165.

Biological activity: Active against gram-positive and gram-negative bacteria. No effect on enterococci, pneumococci, or *Streptococcus viridans*. Active on *Endamoeba histolytica* in rats (2).

Toxicity: Nephrotoxic (2).

References:

1. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
2. Brockmann, H. and Musso, H. Naturwissenschaften **41:** 451–452, 1954.
3. Brockmann, H. and Musso, H. Chem. Ber. **87:** 1779–1799, 1954.
4. Brockmann, H. and Musso, H. Chem. Ber. **88:** 648–661, 1955.
5. Brockmann, H. and Cölln, R. Chem. Ber. **92:** 114–130, 1959.

Granaticin

Produced by: A variety of *Streptomyces olivaceus* which produces a soluble pigment.

Method of extraction: Broth-filtrate adjusted to pH 3 with dilute HCl. Extracted with ethyl acetate. Extract concentrated *in vacuo*. Addition of petroleum ether precipitates crude granaticin. Purified by chromatography on a cellulose column. Cellulose suspended in benzol-saturated formamide; column pretreated with a benzol solution of 8-hydroxyquinoline, followed by washing with formamide-saturated benzol. A benzol solution of crude granaticin applied to column. Elution with formamide-saturated benzol. Four pigmented substances, brown-red, violet-blue (granaticin), blue, and orange are distinguished, in that order. Eluate concentrated to dryness, dissolved in ethyl acetate, and precipitated with petroleum ether. Recrystallization from acetone.

Chemical and physical properties: Tricyclic tetrahydroxyquinone dicarboxylic acid. Small granular red crystals; m.p. 204–206°C (decomposition). Indicator properties: red at acid pH, blue at alkaline pH. $C_{22}H_{20}O_{10}$: C = 59.40%; H = 4.72%; O = 35.95%; C—CH_3 = 7.31%. Crystallographic data given in reference 1. Kuhn-Roth oxidation yields more than 1 mole of acetic acid. Ultraviolet absorption spectrum maxima (rectified alcohol): 223, 286 (496), 532, and 576 mμ. Infrared spectrum given in reference 1. On acetylation gives a tetraacetyl derivative, m.p. 242–243°C (gas generated over 250°C). $[\alpha]_D^{20} = +100°$ (c = 0.818 per cent in chloroform). Absorption spectra given in reference 1.

Biological activity: Active on gram-positive bacteria and *Trichomonas foetus*. Slightly active on *Pasteurella pestis*, *Vibrio comma*, *M. tuberculosis*, and *Endamoeba histolytica*.

Toxicity: 250 mg per kg subcutaneously is lethal for mice.

Reference: 1. Corbaz, R. *et al.* Helv. Chim. Acta **40:** 1262–1269, 1957.

Grasseriomycin

Produced by: Streptomyces lavendulae (1) and *S. griseolavendus* (2).

Method of extraction: Adsorbed from broth-filtrates on charcoal at alkaline pH. Eluted with 80 per cent methanol at pH 2.0. Eluate concentrated *in vacuo*, taken up in anhydrous methanol, and precipitated with acetone. Purified by adsorption on an ion exchange resin, and by salt conversion.

Chemical and physical properties: Basic substance belonging to the streptothricin group. *Base:* Soluble in water, slightly soluble in methanol and ethylene glycol. Insoluble in other organic solvents. No specific absorption in ultraviolet light. Infrared spectrum given in reference 2. Positive Molisch, Fehling, Tollen, and ninhydrin tests. Negative biuret, Sakaguchi, xanthoproteic, Millon, Adamkiewicz, Liebermann, Neubauer, and $FeCl_3$ tests. *Hydrochloride:* Faintly yellow powder. Soluble in water and methanol; insoluble in organic solvents. Molecular weight, 610. *Helianthate:* m.p. 215–225°C (decomposition). *Reineckate:* m.p. 187–190°C (decomposition). C = 22.04%; H = 4.51%; N = 19.35%; Cr = 11.22%. No figures given for S.

Biological activity: Active on gram-negative and gram-positive bacteria, but not on cultures of *E. coli* resistant to streptothricin, neomycin, or grisein. Active on fungi and *Sacch. cerevisiae*, but not on other yeasts. Delays death of silkworms infected with silkworm jaundice virus (1, 2).

Toxicity: Silkworms tolerate injections of 5 μg (2).

References:

1. Ueda, K. *et al.* J. Antibiotics (Japan) **8A:** 91–95, 1955.
2. Sumiki, Y. *et al.* Japanese Patent 6296, August 15, 1957.

Grisamine

Produced by: Streptomyces griseoflavus.

Method of extraction: Broth-filtrate adjusted to

pH 7.4 and stirred with 4 per cent acidic clay. Clay filtered off and eluted with methanol-acetone (1:1). Eluate concentrated *in vacuo*. Extraction of residue with ethyl acetate at pH 7.5 to 8.0. The solvent layer extracted with acidic water and back-extracted with ethyl acetate at an alkaline reaction. The solvent extract is passed through an alumina column and eluted with a mixture of methanol and ethyl acetate (1:1). Concentration of eluate *in vacuo* and extraction of residue with an ethyl acetate-ether (1:10) mixture. Crystallization (needles) occurs in a few hours at room temperature.

Chemical and physical properties: Basic substance. Soluble in water, ethyl acetate, butyl alcohol, and chloroform; slightly soluble in ether; insoluble in petroleum ether, benzene, toluene, and ligroin. Ultraviolet absorption maxima at 255 and 320 mμ in water. Melting point of free grisamine is 165–170°C, and of its sulfate 175–180°C. Negative ninhydrin, biuret, Fehling, Sakaguchi, and $FeCl_3$ tests. Weakly positive *p*-dimethylamine aldehyde and sodium nitroprusside tests. $C_{28}N_6$-$H_{38}O_{10}$: C = 55.62%; H = 6.33%; N = 11.58%.

Biological activity: Active on *M. tuberculosis* strains 607, BCG, and H-2 (human type), and *M. phlei*; very slightly active on *B. subtilis* and *Staph. aureus*. Not active on gram-negative bacteria, yeasts, or fungi tested.

Toxicity: 150 mg per kg intravenously is tolerated by mice; 160 mg per kg causes death within 1 week.

Reference: 1. Sawazaki, T. *et al.* J. Antibiotics (Japan) **8A:** 39–41, 1955.

Grisein

Produced by: Streptomyces griseus strains (1–4, 12) and *S. subtropicus* (8).

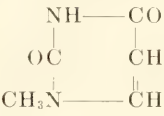
Synonyms: Antibiotic 3510 (3), antibiotic A 1787 (12), albomycin (8, 14, 15). All are identical to, or very closely related to grisein.

Remarks: Zähner *et al.* (15) recently called *sideromycins* antibiotics that show cross resistance with grisein and are antagonized in their action upon gram-positive (but not gram-negative) bacteria by ferrioxamines. They included in this group of antibiotics grisein, albomycin, antibiotic 1787, LA 5352, LA 5937, ferrimycin, and antibiotic 22765; the first three are active upon both gram-positive and gram-negative bacteria and the last four only upon gram-positive bacteria. Ferrioxamines, produced by streptomycetes, form a new group of iron-containing metabolites, with growth-stimulating properties for a number of microorganisms. Bickel *et al.* (16) proposed to include them, together with some known substances, like ferrichrome, coprogen, and the terregens, which either contain or bind iron, in a new class of growth factors, the *sideramines*. This biological property of the sideramines is counteracted by iron-containing antibiotics from streptomycetes, the *sideromycins*.

Method of extraction: Adsorption from broth-filtrate on activated carbon at pH 7.5 to 8.0. Elution with aqueous pyridine, α-picoline, or ethanol (5, 6). Eluate evaporated *in vacuo* and methanol added to precipitate impurities. Addition of ether precipitates grisein. Precipitate leached with methanol, then treated with silver oxide to remove impurities. Distributed between water and phenol-chloroform mixtures by altering the pH of the aqueous phase and the phenol concentration. Reprecipitated from aqueous phase on addition of isopropyl alcohol or ether. Purification by chromatography on silica gel from a solution in 34 per cent phenol-chloroform saturated with pH 4.6, 0.1 *M* citrate buffer; the solution is diluted in half with chloroform before chromatography. Elution with 17 per cent phenol-chloroform (6, 7).

Chemical and physical properties: Complex with neutral or weakly acidic components (6, 11). Red powder (1, 8). Insoluble in ether, chloroform, absolute acetone and ethanol, and benzene. Slightly soluble in acetone and 95 per cent ethanol. Soluble in water and phenol (1, 6). Ultraviolet absorption spectrum maxima at 265 mμ ($E^{1\%}_{1cm}$ 108) and 420 mμ ($E^{1\%}_{1cm}$ 28.9) (6). Positive biuret test (8). Negative Brady, Fehling, Tollen, Liebermann, Sakaguchi, Millon, Pauly, Molisch, Seliwanoff, orcinol, phloroglucinol, and ninhydrin tests (6, 8). Mild deamination results in loss of antibacterial activity (8). Heat-stable (1). Moderately stable to aqueous acid solution; inactivated by methanolic HCl (6). $C_{40}H_{61}N_{10}O_{20}SFe$: N = 13.28%; amino N = 1.34% (6, 8); Fe = 4.50% (11). Molecular weight, 1300 (8, 13) or 1090 (6). Removal of iron causes a loss of color, the ultraviolet peak at 420 mμ, and reduction in biological activity. These changes are reversed on re-addition of Fe to the molecule. Contains four components, A, B, C, and D, with Rf values of 1.0, 0.75, 0.60, and 0.28 on paper chromatography (butanol-acetic acid-water, 4:1:5). Component C breaks down during purification with concomitant appearance of more A (10). Albomycin was first reported to contain three active components and two inactive ones which were degradation products of the first three (11). Electrophoretic studies, however, showed the presence of one major component making up 85 per cent of the complex, and three other trace components (13). Grisein was

reported to form an amorphous picrate, but no helianthate or reineckate (6). Albomycin forms a reineckate (11). No free C=S or =CSH groups. Crude grisein and albomycin hydrolysates contain many amino acids, but purer preparations of grisein contain only glutamic and an unidentified amino acid; purer preparations of albomycin (component D) contain only ornithine and serine (6, 8, 11). An acid hydrolysis product of grisein is 3-methyluracil; m.p. 182–183°C (6):



Biological activity: Active on gram-positive and gram-negative bacteria. No activity on *A. aerogenes*, *B. mycoides*, *Sal. typhosa*, *Pr. vulgaris*, *Ps. aeruginosa*, or fungi. Limited activity on mycobacteria (1, 10). Albomycin is active on *A. aerogenes* (8). Active in mice against *Staph. aureus* and *Sal. schottmuelleri* infections (1). Development of resistance is very rapid (1, 9). Cross-resistance with viomycin. A is the most active component; D the least (10). Inactivated by excess Fe (2, 8), but not by cysteine or hydroxylamine (2).

Toxicity: Nontoxic. Albomycin well tolerated by mice, rabbits, cats, and guinea pigs. No toxic symptoms in clinical use (8).

Utilization: Used clinically in the U. S. S. R. against coccal infections, pneumonia, meningitis, peritonitis, relapsing fever, prostatitis, gonococcal urethritis, and other penicillin-resistant infections (8).

References:

1. Reynolds, D. M. *et al.* Proc. Soc. Exptl. Biol. Med. **64:** 50–54, 1947.
2. Reynolds, D. M. and Waksman, S. A. J. Bacteriol. **55:** 739–752, 1948.
3. Garson, W. and Waksman, S. A. Proc. Natl. Acad. Sci. U. S. **34:** 232, 1948.
4. Umezawa, H. *et al.* J. Antibiotics (Japan) **2B:** 104–109, 1949.
5. Kuehl, F. A., Jr. and Chaiet, L. U. S. Patent 2,505,053, April, 1950.
6. Kuehl, F. A., Jr. *et al.* J. Am. Chem. Soc. **73:** 1770–1773, 1951.
7. Kuehl, F. A., Jr. U. S. Patent 2,546,267, March, 1951.
8. Gause, G. F. Brit. Med. J. **2:**1177–1179, 1955.
9. Garrod, L. P. and Waterworth, P. M. Brit. Med. J. **2:** 61–65, 1956.
10. Stapley, E. O. and Ormond, R. E. Science **125:** 587–589, 1957.
11. Braznikova, M. G. *et al.* Biokhimiya **22:** 111–117, 1957.
12. Thrum, H. Naturwissenschaften **44:** 561–562, 1957.
13. Samsonov, G. V. *et al.* Biokhimiya (Eng. transl.) **23:** 206–209, 1958.
14. Waksman, S. A. Science **125:** 585–587, 1957.
15. Zähner, H. *et al.* Arch. Mikrobiol. **36:** 325 349, 1960.
16. Bickel, V. H. *et al.* Experientia **16:** 129–133, 1960.

Griseoflavin

Produced by: Streptomyces griseoflavus.

Method of extraction: Adsorption on acidic clay at pH 2.0, elution with 80 per cent acetone at pH 7.0. Evaporation of acetone, aqueous solution extracted with ethyl acetate, evaporation to dryness. Brown powder dissolved in methanol, chromatography over aluminum oxide. Crystallization from ethanol.

Chemical and physical properties: Colorless crystals; m.p. 210–215°C (decomposition). Soluble in methanol, ethanol, propanol, phenol, acetic acid, and alkaline water. Slightly soluble in water, ethyl acetate, and butyl acetate. Insoluble in ether, petroleum ether, benzene, and chloroform. Negative biuret, ninhydrin, Sakaguchi, Molisch, and $FeCl_3$ tests.

Biological activity: Active primarily against gram-positive bacteria; limited activity against gram-negative bacteria and mycobacteria. Strains of staphylococci, *Vibrio comma*, and one *Mycobacterium* are the most sensitive organisms (1 to 2 μg per ml).

Toxicity: LD_{50} (mice) >250 mg per kg intraperitoneally.

Reference: 1. Waga, Y. J. Antibiotics (Japan) **6A:** 66–72, 1953.

Griseolutein A

Produced by: Streptomyces griseoluteus (3).

Synonym: Griseolutein. The same organism also produces griseolutein B.

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 2.0. Extract concentrated *in vacuo*. Concentrate chromatographed on alumina and developed with ethyl acetate. Active yellow fraction concentrated and cooled to precipitate crystals. Recrystallized from ethyl acetate (1). Crude powder containing both A and B is dissolved in aqueous sodium bicarbonate (pH 7.2) and solution extracted with ethyl acetate at pH 5.8. Most of B remains in the aqueous layer; A is

transferred to the solvent. Solvent concentrated *in vacuo*. A is purified by countercurrent distribution (ethyl acetate-phosphate buffer, pH 5.8). Crystallized from ethyl acetate. Recrystallized from methanol (6).

Chemical and physical properties: Orange-yellow needles; m.p. 194–197°C (decomposition). Ultraviolet absorption spectrum maxima at 265 mμ ($E^{1\%}_{1cm}$ 1980) and 362 mμ ($E^{1\%}_{1cm}$ 330) (methanol). Infrared spectrum given in reference 6. $C_{17}H_{14}O_6N_2$: C = 59.51%; H = 4.36%; N = 8.02%; O—CH_3 = 7.3%. Insoluble in water, ether, and benzene. Slightly soluble in ethyl acetate and ethanol. Soluble, but destroyed, at alkaline reaction. More stable at pH 2.0 than 7.0. *Monomethyl ester of A:* Yellow crystals; m.p. 149°C. The structure of griseolutein A, 1-methoxy-4-[(hydroxyacetoxy)methyl]-9-carboxyphenazine (6, 7), is given in Chapter 6.

Biological activity: Active on gram-positive and gram-negative bacteria (1). Activity affected by cysteine and serum, but not by cystine. More active at acid than alkaline pH (2, 4).

Toxicity: >2 gm per kg or >500 mg per kg are not toxic to mice subcutaneously (2, 4).

References:

1. Umezawa, H. *et al.* J. Antibiotics (Japan) **4:** 34–40, 1951.
2. Ogata, Y. J. Antibiotics (Japan) **4:** 213–218, 1951.
3. Okami, Y. J. Antibiotics (Japan) **5:** 477–480, 1952.
4. Ogato, T. *et al.* J. Antibiotics (Japan) **7A:** 15–16, 1954.
5. Nakamura, S. *et al.* J. Antibiotics (Japan) **10A:** 265–266, 1957.
6. Nakamura, S. *et al.* J. Antibiotics (Japan) **12A:** 55–58, 1959.
7. Yagishita, K. J. Antibiotics (Japan) **13A:** 83–96, 1960.

Griseolutein B

Produced by: A mutant of the *Streptomyces griseoluteus* culture that produces griseolutein A.

Method of extraction: Broth-filtrate extracted with butyl acetate at pH 2.0. Extract concentrated *in vacuo* to precipitate griseolutein B. Crude powder dissolved in dioxane, then concentrated to give crystalline substance (2, 4). Purified by countercurrent distribution between pH 5.8 phosphate buffer and ethyl acetate. Acidification of active fractions gives crystals. Recrystallized from pyridine-dioxane-ether (7).

Chemical and physical properties: Contains a phenazine nucleus (6). Yellow prisms. Browns at about 160°C, darkens at 180°C, and chars at 220°C. Soluble in aqueous $NaHCO_3$ and pyridine. Sparingly soluble in dioxane and methyl ethyl ketone. Insoluble in toluene, ether, and butyl acetate. Ultraviolet absorption spectrum maxima at 281 to 283 mμ ($E^{1\%}_{1cm}$ 296) and 342 to 344 mμ ($E^{1\%}_{1cm}$ 170) (methanol). Infrared absorption spectrum given in reference 1. $[\alpha]_D^{23} = -6°$ (c = 0.7 per cent in pyridine) (7). Infrared spectrum given in reference 4. Unstable in aqueous solution. $C_{47}H_{16}O_6N_2$ (3). The structure of griseolutein B (1-methoxy-4(α,β-dihydroxyethoxymethyl)phenazine - 9 - carboxylic acid) (8–10) is given in Chapter 6. *Diacetyl griseolutein B:* Light yellow prisms (7). Alkaline hydrolysis of this derivative gives griseoluteic acid (7).

Biological activity: Active on gram-positive and gram-negative bacteria, mycobacteria, and protozoa (2, 3, 5). Antirickettsial activity (3). Only slight cross-resistance between A and B. Active in mice on *Staph. aureus* infections (2). Active on Ehrlich carcinoma in mice (5). B has weaker antibacterial activity than A, especially against *Pr. vulgaris* (4). Diacetyl griseolutein B is biologically active (8).

References:

1. Yagishita, K. *et al.* J. Antibiotics (Japan) **6A:** 113–116, 1953.
2. Ogata, Y. *et al.* J. Antibiotics (Japan) **6A:** 139, 1953.
3. Ogata, Y. Japan. J. Med. Sci. & Biol. **6:** 493–501, 1953.
4. Osato, T. *et al.* J. Antibiotics (Japan) **7A:** 15–16, 1954.
5. Umezawa, H. Giorn. microbiol. **2:** 160–193, 1956.
6. Nakamura, S. *et al.* J. Antibiotics (Japan) **10A:** 265–266, 1957.
7. Nakamura, S. Chem. Pharm. Bull. **6:** 539–543, 1958.
8. Nakamura, S. Chem. Pharm. Bull. **6:** 547–550, 1958.
9. Nakamura, S. J. Antibiotics (Japan) **12A:** 26–27, 1959.
10. Yagashita, K. J. Antibiotics (Japan) **13A:** 83–96, 1960.

Griseomycin

Produced by: Streptomyces griseolus (1, 3).

Synonyms: Griseomycin is similar or identical to proactinomycin B, and is isomeric with picromycin (6). Related to amaromycin (1) and lomycin (2).

Method of extraction: Whole culture acidified to pH 2.0 and filtered. Broth-filtrate can be extracted

at an alkaline pH by most organic solvents (benzene, chloroform, ether, amyl acetate, and toluene). Back-extraction in water at pH 2.0. After two or three purifications in this manner, concentration of organic solvent solution permits crystallization (1, 3).

Chemical and physical properties: Macrolide, containing desosamine. White plate crystals, having a bitter taste; m.p. 76–80°C (1) or 70–75°C (3). Very soluble in most organic solvents, but only slightly soluble in water. Infrared data given in reference 3. $[\alpha]_D^{25} = +32°$ (c = 1 per cent in $CHCl_3$) (1), or $[\alpha]_D = -7.0°$ ($CHCl_3$) (3). Stable at room temperature between pH 2 and 9, but rapidly destroyed at 100°C. $C_{28}H_{48}NO_8$. Molecular weight, 530. *Hydrochloride:* m.p. 120°C. Very soluble in water, ethanol, methanol, and chloroform. Poorly soluble in other organic solvents. Precipitated from aqueous solutions by picric acid and Reinecke salt (1, 3, 6). Griseomycin reacts with methyl iodide to form a quaternary salt: $C_{29}H_{51}NO_8I$, m.p. 193–195°C. $[\alpha]_D = +31.0°$ (ethanol). This salt has the same formula, melting point, and infrared spectrum as the pieromycin quaternary salt formed with methyl iodide, but differs in rotation (5).

Biological activity: Active mainly against gram-positive bacteria, including mycobacteria. Active against *Neisseria*. Inactive against most gram-negative bacteria and *C. albicans*. Cross-resistance *in vitro* between carbomycin, erythromycin, and griseomycin. Not inactivated *in vitro* by human serum or whole blood (1). Destroyed in the liver (4).

Toxicity: LD_{50} (mice) 1330 mg per kg subcutaneously, 210 mg per kg intraperitoneally. Orally, 2100 mg per kg is not toxic. Griseomycin is absorbed by the gastrointestinal wall (1). Nontoxic to rabbits when applied cortically (6).

References:

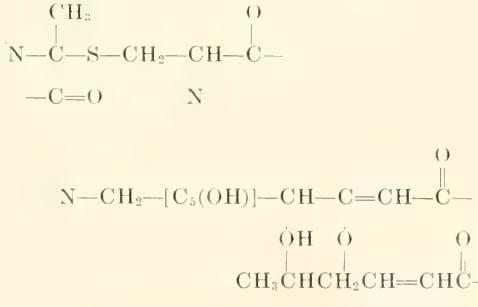
1. Van Dijck, P. J. *et al.* Antibiotics & Chemotherapy **3:** 1243–1246, 1953.
2. DeSomer, P. *et al.* Resumés Communs. 6th Intern. Congr. Microbiol. **1:** 161, 240, 1953.
3. Belgian Patent 522,647, September 30, 1953.
4. DeSomer, P. *et al.* Antibiotics & Chemotherapy **4:** 546–550, 1954.
5. Vanderhaeghe, H. Cited in reference 4.
6. DeSomer, P. Giorn. microbiol. **2:** 216–232, 1956.

Griseoviridin

Produced by: Streptomyces griseus and *S. griseoviridus* (1, 5). These same strains also produce etamycin (viridogrisein) (1).

Method of extraction: Culture-broth adjusted to pH 4.4 and filtered on Hyflo Super-Cel. Extraction of the filtrate at pH 7.2 with 0.5 volume of ethylene dichloride. Concentration of the extract *in vacuo* yields a precipitate of crude griseoviridin, which is added to boiling absolute methanol and filtered on paper. To the filtrate, Nuchar C190N is added; after refluxing for 5 minutes, the suspension is filtered through paper. Crystallization is allowed to proceed by storing the filtrate at 8°C. The crystals are washed with cold absolute methanol and dried *in vacuo* (1). Crystallized from pyridine (2).

Chemical and physical properties: Neutral substance. *Griseoviridin base:* Plate crystals; m.p. 228–230°C. Soluble in pyridine; moderately soluble in lower alcohols; sparingly soluble in water and nonpolar solvents. Ultraviolet absorption spectrum maximum at 220.5 mμ (ε = 44,000) and an inflection at 277.5 mμ (ε = 1500). Infrared data given in reference 2. $[\alpha]_D^{27} = -237°$ (c = 0.5 per cent in methanol). Contains no thiol, methoxyl, or methylimino groups. Positive Baeyers and chromate-nitric acid tests. Negative $FeCl_3$, Folin-Ciocalteau, Sakaguchi, and Jacobs-Hoffman tests. Acid hydrolysis products include cysteine, serine, and an unidentified ninhydrin-positive substance (3). $C_{22}H_{29}O_7N_3S$: C = 54.9%; H = 5.8%; O = 24.4%; N = 8.7%; S = 6.2%. Molecular weight, 485 to 490 (1–3). Griseoviridin crystallizes from methanol in two different forms, depending on the temperature of the solvent during crystallization. Form A (a hemimethanolate) is obtained at >30°C; form B at <30°C. B loses solvent in air to give A. *Form A:* Large crystals; m.p. 160°C. $[\alpha]_D^{27} = -237°$ (c = 0.5 per cent in methanol). Crystallographic data given in reference 2. *Reaction product with HCl:* Needles; m.p. about 180°C (decomposition) (3). Partial structure of griseoviridin (4):



Biological activity: Active *in vitro* against a variety of gram-positive and gram-negative bacteria and actinomycetes at a level of 0.1 to 10 μg

per ml, such as strains of *A. bovis*, *Br. suis*, *Clostridium hemolyticum*, *Corynebacterium diphtheriae*, *D. pneumoniae*, *E. coli*, *H. influenzae*, *Neisseria catarrhalis*, *Sh. paradysenteriae*, and *Streptococcus pyogenes*. Among the nonsensitive bacteria are strains of *K. pneumoniae*, *Staph. aureus*, *Pr. vulgaris*, and *Ps. aeruginosa*. No activity against fungi, *Endamoeba histolytica*, *Trichomonas foetus*, *Trypanosoma cruzi*, or *T. rhodesiense*. Active against *H. pertussis* in mice. Not active against experimental tuberculosis of mice. Active against bovine mastitis caused by *Staph. aureus*, *Staph. albus*, or *E. coli*, and against infectious bronchitis of chickens. Limited antirickettsial activity against *Miyagawanella ornithosis* and *M. psittacii* in mice, and *Rickettsia prowazekii* in eggs. Inactive against viruses in embryonated eggs (1).

Toxicity: LD_{50} (mice) 75 mg per kg intravenously, >100 mg per kg intraperitoneally, >100 mg per kg subcutaneously. Maximal tolerated dose (mice) 50 mg per kg intraperitoneally. Not irritating when instilled into the udders of lactating cows (1).

References:

1. Bartz, Q. R. *et al.* Antibiotics Ann. 777–805, 1954–1955.
2. Ames, D. E. *et al.* J. Chem. Soc. 4260–4263, 1955.
3. Ames, D. E. and Bowman, R. E. J. Chem. Soc. 4264–4270, 1955.
4. Ames, D. E. and Bowman, R. E. J. Chem. Soc. 2925–2928, 1956.
5. Anderson, L. E. *et al.* Antibiotics & Chemotherapy **6:** 100–115, 1956.

Grizin

Produced by: Streptomyces sp. of the *S. griseus* group (1).

Synonyms: Grizemin, antibiotic IEM I.

Method of extraction: Broth treated with charcoal at pH 3. Filtrate adjusted to pH 4.5, refiltered, and antibiotic adsorbed at pH 7.5. Elution with 40 to 50 per cent ethanol at pH 3. Neutralization followed by concentration *in vacuo*. Purified by formation of various crystalline salts, *e.g.*, picrate and helianthate (1).

Chemical and physical properties: Basic antibiotic. *Helianthate:* Brown powder; m.p. 194–196°C (decomposition). *Hydrochloride:* White powder. Soluble in water and methanol. Stable as dry powder and to boiling for 10 minutes. Positive biuret, ninhydrin, and Bertrand tests. Test for glucosamine positive. Negative Sakaguchi, maltol, and histidine tests. N = 13.6 to 14.9% (helianthate) in the form of α-amino nitrogen (1). Probably a polypeptide.

Biological activity: Active on gram-negative and gram-positive bacteria, yeasts, and fungi. Active on dysentery in mice. Effective on angular leaf spot of cotton, and lemon and mandarin necrosis (*Bacterium citriputeale*). Prevents withering of apricot (*Bact. armeniaca*). Effective against a lemon tree disease (*Deuterophoma tracheiphilla*) (1).

Toxicity: Mice tolerate 0.5 mg per day for 6 days (1).

Utilization: Dysentery in children (1).

Reference: 1. Krassilnikov, N. A. *et al.* Mikrobiologiya **26:** 418–425, 1957.

Grubilin

Produced by: Streptomyces sp.

Method of extraction: Isolated from the mycelium with organic solvents.

Chemical and physical properties: Green-yellow substance. *Base:* Insoluble in water and organic solvents. Gives color reactions and an ultraviolet absorption spectrum typical of a heptaene. *Na salt:* Water-soluble.

Biological activity: Active on yeasts and filamentous fungi; not active on bacteria or *Streptomyces*.

Toxicity: LD_{50} (mice) 15 mg per kg intravenously, 30 mg per kg intraperitoneally, and >500 mg per kg subcutaneously.

Reference: 1. Uri, J. *et al.* Abstr. Communs. Symposium on Antibiotics, Prague. 46–47, 1959.

Heliomycin

Produced by: Streptomyces flavochromogenes var. *heliomycini*.

Synonyms: Resistomycin, antibiotic X 340.

Method of extraction: Mycelium washed and pressed. Extracted with 3 volumes of technical acetone per volume of wet mycelium. Extraction repeated with one half the volume of acetone. To the combined acetone-extracts, saturated barium hydroxide is added until no further precipitation occurs. The precipitated barium salt of heliomycin is suspended in water. HCl added to bring the pH to 3.0; this solubilizes barium as barium chloride, which is removed by filtration. The precipitated heliomycin is put in solution in acetone at pH 7.5 and impurities filtered off. Precipitation of yellow crystalline heliomycin upon acidification to pH 3.0 with HCl. Recrystallization from acetone or dioxane.

Chemical and physical properties: Yellow needle-shaped crystals. Soluble in dioxane, acetone, ethyl acetate, butyl and ethyl alcohols. Less soluble in chloroform, ether, and benzene. Poorly soluble in carbon tetrachloride. The solubility in organic

solvents is increased by acidification. Insoluble in water unless strong alkalies are added; then it gives a red color. Very stable except in aqueous alkaline solutions, such as 0.1 *N* NaOH. No melting point; upon heating over 100°C, it decomposes. Molecular weight, 235 (Rast). Positive Millon, sodium nitrite, and sodium nitroprusside tests. Positive Anchel test for phenols and quinones. No reduction of Fehling's reagent. Contains no N, S, or P. Maximal absorption of light at 269, 290, 320, 340, 370, 460, and 515 mμ (alcoholic solution). Alkaline degradation is accompanied by changes in light-absorption spectrum (given in reference 4).

Biological activity: Active on staphylococci and influenza virus (contact test). Slight activity on influenza in mice (1). Active on tobacco mosaic virus *in vitro* (2) and smallpox vaccine (4). *B. mycoides* used as assay organism (5).

Toxicity: LD_{50} (mice) 25 mg per kg intraperitoneally (6).

References:

1. Gause, G. F. Giorn. microbiol. **2:** 194–200, 1956.
2. Ukholina, R. S. Mikrobiologiya **27:** 347–350, 1958.
3. Brajhnikova, M. G. *et al.* 2nd All-Union Conf. Antibiotics, Moscow, p. 9, 1957.
4. Kremer, V. E. Antibiotiki **4(6):** 59–63, 1959.
5. Brajhnikova, M. G. *et al.* Antibiotiki **3(2):** 29–34, 1958.
6. Goldberg, L. E. Antibiotiki **5(1):** 107–112, 1960.

Holomycin

Produced by: Streptomyces griseus.

Synonym: Belongs to the thiolutin-aureothricin series, differing from thiolutin only in the $N—CH_3$ group.

Method of extraction: Broth-filtrate extracted with ethyl acetate. Extract concentrated *in vacuo*. Chromatographed on aluminum oxide, washed with absolute chloroform and chloroform-methanol (99:1), and eluted with chloroform-methanol (97:3). Active fractions concentrated *in vacuo* to an oily residue. Residue taken up in ethyl acetate; the antibiotic precipitates after a short time. Recrystallized from methanol-ethyl acetate.

Chemical and physical properties: Des-N-methylthiolutin. Neutral. Glittering orange-yellow rhombic crystals; m.p. 264–271°C (mixed melting point with thiolutin, 240–250°C). Could be sublimed *in vacuo*. Soluble in organic solvents. Ultraviolet absorption spectrum maxima (ethanol) at about 390 mμ, with lesser peaks at about 303 and 250 mμ. Infrared spectrum given in reference 1. Paper chromatographic data given in reference 1. $C_7H_6O_2N_2S_2$: C = 39.25%; H = 2.79%; N = 13.07%; S = 29.77%; $C—CH_3$ = 7.04%; CH_3CO = 21.38%. Acid hydrolysis product is "holothin," $C_5H_3ON_2$ or des-N-methylpyrrothine. Structural formula of holomycin given in Chapter 6.

Biological activity: Active on *Streptococcus pyogenes* (1 μg per ml), *V. cholerae*, *M. tuberculosis*, *E. coli*, *Sal. schottmuelleri*, and *Klebsiella* (10 μg per ml); *Staph. aureus*, *Ps. aeruginosa*, *C. albicans*, and *Endomyces albicans* (101 μg per ml). Active on *Trichomonas foetus* at 1 μg per ml and *Endamoeba histolytica* at 10 μg per ml. Holothin has some antibiotic activity.

Reference: 1. Ettlinger, L. *et al.* Helv. Chim. Acta **42:** 563–569, 1959.

Humidin

Produced by: Streptomyces humidus, the strain that also produces dihydrostreptomycin.

Method of extraction: Cells extracted with acetone. Concentration of solvent under reduced pressure. Aqueous concentrate acidified with HCl and extracted with ethyl acetate. Back-extraction with 1 *N* NaOH. Upon neutralization, crystallization occurs.

Chemical and physical properties: Colorless platelets; m.p. 145–146°C. Tentative empirical formula: $(C_{12}H_{20}O_4)_n$. $[\alpha]_D^{34} = -6°$ (c = 1 per cent in ethanol). $[\alpha]_D^{25} = -10°$ (c = 1 per cent in acetone) and −8° (c = 1 per cent in dioxane). Light-absorption maxima at about 245 and 285 mμ. Infrared absorption spectrum given in reference 1. Soluble in acetone, dioxane, and ethyl acetate. Slightly soluble in n-butyl alcohol, ether, and ethanol. Insoluble in methanol, benzene, water, petroleum ether, and carbon tetrachloride. Orange color with sulfuric acid. Potassium permanganate and bromine water decolorized. Negative Fehling reaction.

Biological activity: Active against certain fungi and protozoa. No activity against bacteria. Active against *Saccb. cerevisiae* but not against species of *Candida*. More active at alkaline than at acid pH. Activity reduced by ascorbic acid but not by cysteine.

Toxicity: LD_{50} (mice) 4.5 mg per kg intraperitoneally, 54 mg per kg orally.

Reference: 1. Nakazawa, K. *et al.* J. Agr. Chem. Soc. Japan **32:** 713–716, 1958.

Hydroxymycin

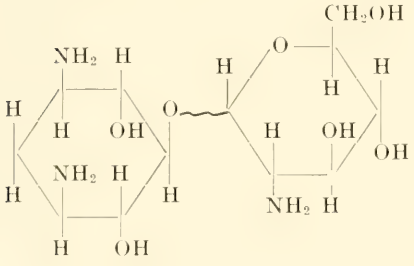
Produced by: Streptomyces paucisporogenes (2).

Synonyms: Antibiotic 4915 (2). Similar to catenulin and paromomycin (4).

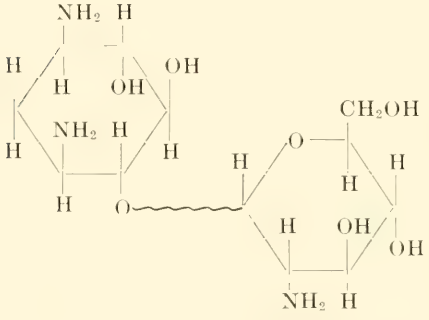
Method of extraction: Culture-filtrate adjusted

to pH 6 to 7, calcium precipitated as the oxalate, and the whole filtered. Filtrate adsorbed on IRC-50 (Na^+ phase), eluted with 1 *N* H_2SO_4, and precipitated as the pentachlorophenol derivative. Purified by conversion to the sulfate, followed by fractional crystallization from an aqueous methanolic solution of the *p*-(*p'*-hydroxyphenylazo)benzene sulfonate (2).

Chemical and physical properties: Basic amino polysaccharide. *Base:* White amorphous powder. Soluble in water and methanol. Insoluble in common organic solvents. $[\alpha]_D^{20} = +63° \pm 2°$ (c = 1 per cent in water). $C_{25}H_{47}O_{15}N_5$. Molecular weight, 610 (2, 3). Methanolysis in the presence of HCl gives a product which was termed "pseudoneamine," $C_{12}H_{25}O_7N_3$, composed of 1,3-diamino-4,5,6-trihydroxycyclohexane (meso) (also present in neomycin and kanamycin) and D-glucosamine. Two possible structures for pseudoneamine were proposed:



or



Sulfate: White amorphous powder. No characteristic infrared or ultraviolet absorption spectrum. $[\alpha]_D^{20} = +50–52°$ (c = 1 per cent in water) and does not vary with pH. Rf = 0.46 on paper chromatography (methanol-2 per cent aqueous NaCl, 2:1), paper impregnated with $NaHSO_4$ at pH 2.4. Positive ninhydrin, diazo *p*-nitroaniline (for primary amino groups), and periodic acid tests. Negative nitroprusside (secondary amino groups), Fehling, Sakaguchi, Pauly, and Elson-Morgan tests. Furfural formed on treatment with acid. Total N = 6.2%; N (Van Slyke) = 6.0%; N (Sorensen) = 3%. *p-(p'-Hydroxyphenylazobenzene sulfonate:* Orange crystals; m.p. 220°C (decomposition). Thermostable from pH 2 to 10. $[\alpha]_D^{20} = +37°$ (c = 0.5 per cent in methanol) (2). *N-Benzoyl hydroxymycin:* White needles; m.p. 232°C (decomposition). $[\alpha]_D^{20} = +36° \pm 2°$ (c = 0.3 per cent in methanol). Biologically inactive (2).

Biological activity: Active on gram-negative and gram-positive bacteria and mycobacteria. Active on *Endamoeba histolytica* at 5 to 10 μg per ml (2). Weak activity *in vitro* on *Trichomonas vaginalis* (1). Not affected by serum or glucose. Active on mycobacteria resistant to streptomycin. Active in mice on infections caused by *Staph. aureus, Diplococcus mucosus, K. pneumoniae, Sal. typhosa, E. coli,* and *Ps. aeruginosa.* Less active on *D. pneumoniae* and *Streptococcus hemolyticus* infections. As active as streptomycin on tuberculosis in mice and guinea pigs (2). Active in mice on *T. vaginalis* (1). No antiviral activity *in vivo.*

Toxicity: LD_{50} (mice) 125 ± 5 mg per kg intravenously, 1020 ± 110 mg per kg subcutaneously. Does not have neomycin-like toxicity (2).

References:

1. Vaisman, A. and Hamelin, A. Compt. rend. **247:** 163–165, 1958.
2. Hagemann, G. *et al.* Ann. pharm. franç. **16:** 585–596, 1958.
3. Bartos, M. J. Ann. pharm. franç. **16:** 596–600, 1958.
4. Schaffner, C. P. Personal communication.

Hydroxystreptomycin

Produced by: Streptomyces rubrireticuli (formerly referred to as *S. reticuli*) (1, 11). This strain also produces rotaventin. *S. griseocarneus* (2, 3, 5, 6, 8). These strains also produce an antifungal substance in low yield (9). *Streptomyces* sp. This strain differs from the above, but belongs to the *reticuli* group (12).

Synonyms: Reticulin (1, 10), antibiotic NA 232-M1 (2).

Method of extraction: I. See I under streptomycin. II. Culture-broth acidified, filtered, neutralized, and adsorbed on IRC-50. Eluted with 0.5 *N* H_2SO_4. Eluate neutralized, concentrated, and acetone added to give the crude substance. Purified by chromatography on Darco G-60-Celite 545 (2).

Chemical and physical properties: Differs from streptomycin by one oxygen atom present as a hydroxyl group on the methyl group of the streptose moiety (2) (see Chapter 6). Soluble in water, ethanol, and dilute acids (9). $[\alpha]_D = -790°$ (2).

Positive Sakaguchi, Elson-Morgan, Molisch, and Benedict tests (1). Negative biuret, ninhydrin, Fehling, and maltol tests (2). Rf = 0.09 to 0.11 (n-butanol, 2 per cent piperidine, 2 per cent *p*-toluene sulfonic acid monohydrate) (1). About 50 per cent inactivation at 100°C for 5 to 10 minutes (1). *Trihydrochloride:* White substance (4). $[\alpha]_D^{27} = -95°$ (c = 1 per cent in water) (4). C = 35.6%; H = 5.95%; N = 13.9%; Cl = 14.8%. $C_{21}H_{39}N_7O_{13}\cdot 3HCl$. *Helianthate:* Reddish brown crystals. Darken at about 220°C and char without melting. X-ray diffraction pattern similar to streptomycin helianthate (4). Yields a dihydro derivative on catalytic hydrogenation (3, 8). Alkaline hydrolysis products include pyromeconic and isokojic acids, but no maltol (2, 10). Isokojic acid (2-hydroxymethyl-3-hydroxy-1,4 pyrone): m.p. 154–157°C. Ultraviolet absorption spectrum maximum at 274 mμ ($E_{1cm}^{1\%}$ 690) in 0.1 *N* HCl. Positive $FeCl_3$ test (2, 3).

Biological activity: Similar to that of streptomycin (1, 2). Inactivated by cysteine and reactivated by iodine (1).

Toxicity: LD_{50} (mice) 154 mg per kg intravenously, 865 to 948 mg per kg subcutaneously (2, 7). Acute and chronic toxicity said to be essentially the same as for streptomycin.

Utilization: None. Presents no known advantage over streptomycin.

References:

1. Hosoya, S. *et al.* Japan. J. Exptl. Med. **20:** 327–337, 1949.
2. Grundy, W. E. *et al.* Arch. Biochem. **28:** 150–152, 1950.
3. Benedict, R. G. *et al.* Science **112:** 77–78, 1950.
4. Stodola, F. H. *et al.* J. Am. Chem. Soc. **73:** 2290–2293, 1951.
5. Benedict, R. G. *et al.* J. Bacteriol. **62:** 487–497, 1951.
6. Grundy, W. E. *et al.* Antibiotics & Chemotherapy **1:** 309–317, 1951.
7. Ambrose, A. M. Proc. Soc. Exptl. Biol. Med. **76:** 466, 1951.
8. Benedict, R. G. and Stodola, F. H. U. S. Patent 2,617,755, November 11, 1952.
9. Hosoya, S. *et al.* J. Antibiotics (Japan) **5:** 525–527, 1952.
10. Hosoya, S. *et al.* J. Antibiotics (Japan) **6A:** 102, 1953 (abstr. of **6B:** 61–66, 1953).
11. Hosoya, S. Quoted in Benedict, R. G. Botan. Rev. **19:** 229–320, 1953.
12. Nakazawa, K. *et al.* Ann. Rept. Takeda Research Lab. **13:** 67–77, 1954.

Hygromycin

Produced by: Streptomyces hygroscopicus (1) and *S. noboritoensis*. The latter organism also produces blastmycin and an antibiotic active on gram-positive bacteria (7).

Synonym: Homomycin (7).

Method of extraction: I. Broth-filtrate saturated with $(NH_4)_2SO_4$ and extracted with n-butanol. Extract concentrated *in vacuo*, filtered, and petroleum ether added to precipitate hygromycin. Chromatographed on carbon from a 0.001 *N* sulfuric acid solution and developed with an aqueous solution containing 10 per cent n-butanol and 30 per cent acetone. Active fractions concentrated *in vacuo* with addition of n-butanol to remove water. Antibiotic precipitated on addition of petroleum ether. Further purified by countercurrent distribution between n-butanol or n-amyl alcohol and water-glacial acetic acid (2). II. Culture media acidified, stirred with acidic clay, and filtered. Filtrate neutralized and stirred with active carbon. Eluted from the carbon with 80 per cent aqueous acetone. Eluates concentrated *in vacuo* and acetone added to the concentrate to precipitate impurities. Concentrated *in vacuo*, then lyophilized. Chromatographed on alumina from methanol-ethanol (1:1) and developed with acetone containing 20 per cent 0.5 *N* HCl. Further purification by chromatography on silica gel with water-saturated n-butanol as solvent and developer. Active fractions treated with carbon, concentrated *in vacuo*, and precipitated by adding ether or petroleum ether. Final purification by countercurrent distribution, first in an n-butanol-0.25 *M* phosphate buffer (pH 4.6), then in n-butanol-ethyl acetate-water (1.2:0.5:1.9) (7).

Chemical and physical properties: Weakly acidic substance (2). White powder. Gradually melts at 80–90°C. Colors above 155°C (7). Very soluble in water and ethanol; essentially insoluble in less polar solvents. Infrared absorption spectrum given in reference 2. Ultraviolet absorption spectrum maxima in dilute acid at 214 mμ ($E_{1cm}^{1\%}$ 416) and 272 mμ ($E_{1cm}^{1\%}$ 306) (2); in dilute alkali at 254 mμ ($E_{1cm}^{1\%}$ 350), 286 mμ ($E_{1cm}^{1\%}$ 194), and 323 mμ ($E_{1cm}^{1\%}$ 116) (6); in water at 270 to 272 mμ ($E_{1cm}^{1\%}$ 291) (3). $[\alpha]_D^{25} = -126°$ (c = 1 per cent in water) (2). Positive Folin-Ciocalteau, Fehling, Benedict, diazo, iodoform, Nessler, Tollen, indole, and carbazole tests. Negative $FeCl_3$, Ehrlich, ninhydrin, biuret, phloroglucinol, Seliwanoff, glucosamine, anthrone, Molisch (doubtful), and maltol tests (2, 3, 7, 8). Acetylation product is biologically inactive (2, 3). $pK_a' = 8.9$ (water) (6). Rf = 0.63 in water-saturated n-butanol. Cannot be hydro-

genated; yields a biologically inactive tetraacetate (7). Stable to boiling for 10 minutes at pH 3.7; less stable at pH 9.0 and above (1). *2,4-Dinitrophenylhydrazone derivative:* Yellow crystalline substance; m.p. 154–156°C (6, 7). Acid hydrolysis products of hygromycin include neo-inosamine-2 (5, 6). Alkaline hydrolysis yields 3,4-dihydroxy-α-methylcinnamic acid. The sugar in hygromycin is 5-keto-6-desoxyarabohexose, $C_{25}H_{29}NO_{12}$: C = 53.85%; H = 6.15%; N = 2.78%; O = 37.53%; C—Me = 2.56%. The possible structure of hygromycin (6) is discussed in Chapter 6.

Biological activity: Active on certain gram-positive and gram-negative bacteria, mycobacteria, and actinomycetes at 3 to 51 μg per ml (1). Active on *Endamoeba histolytica* and *Leptospira pomona* (11). Very active on pleuropneumonia-like organisms (9). Activity on *K. pneumoniae* inhibited by cysteine. Activity unaffected by pH. Rate at which resistance develops depends on the microorganism used. Active in mice on *M. tuberculosis* H37Rv, but only one third to one fifth the activity of streptomycin. Also active in mice on *Streptococcus pyogenes* and *Borrelia novyi*, and moderately active on *K. pneumoniae*. Also active against mouse meningopneumonitis infections. Not active against viruses such as "MM" and Semiliki Forest viruses (1, 7). Active on *E. histolytica* (rats) and oxyurids (mice) (11).

Toxicity: Mice tolerate 2 gm per kg intravenously and subcutaneously (7).

Utilization: Prevention of bacterial decomposition of fish stickwater (4). Used in animal feeds for prevention and cure of large round worms, nodular worms, and whipworms (10).

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2. Mann, R. L. *et al.* Antibiotics & Chemotherapy **3:** 1279–1282, 1953.
3. Sumiki, Y. *et al.* J. Antibiotics (Japan) **8A:** 170, 1955.
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11. McCowen, M. C. *et al.* Antibiotics Ann. 883–886, 1956–1957.
12. Handy, A. H. *et al.* Poultry Sci. **36:** 748–754, 1957.

Hygromycin B

Produced by: Streptomyces hygroscopicus.

Method of extraction: Broth treated with Amberlite IRC-50 (Na^+ cycle). Elution with 0.1 *N* HCl. Eluate treated with carbon at pH 10.5. Hygromycin B eluted with a mixture of concentrated NH_4OH, water, and acetone (1:3:6). Eluate concentrated and antibiotic precipitated with acetone. Precipitate dissolved in methanol. Reprecipitation with ether. Further purification by adsorption on Amberlite IRC-50 (Li^+ cycle) and elution with 29 per cent NH_4OH (1).

Chemical and physical properties: Polyhydroxy base. Amorphous powder. Melts over wide range about 180°C. $C_{15}H_{28}N_2O_{9-10}$. Very soluble in water and methanol; essentially insoluble in less polar solvents. Two titrable basic groups: pK_a' 7.1 and pK_a' 8.8. No absorption of ultraviolet light. Infrared data given in reference 1. Positive anthrone and Molisch tests. Negative Benedict and Fehling tests. Forms a crystalline *p*-(*p'*-hydroxyphenylazo)benzenesulfonic acid salt.

Biological activity: Moderate activity on gram-positive and gram-negative bacteria and fungi (6.2 to 100 μg per ml). Very active on helminths, including ascarids, in swine (1). Increases growth rate of baby pigs when added to the diet (2).

Utilization: Anthelmintic.

References:

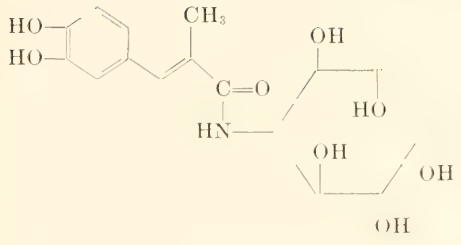
1. Mann, R. L. and Bromer, W. W. J. Am. Chem. Soc. **80:** 2714–2716, 1958.
2. Teague, H. S. and Rutledge, E. A. Ohio Agr. Expt. Sta. Mimeo 114, July, 1959.

Hygromycin-like Antibiotic

Produced by: Streptomyces sp.

Synonyms: Antibiotic 1703-18B. Similar, but not identical, to hygromycin (1).

Chemical and physical properties: Acid hydrolysis products include: neo-inosamine-2 (1) and the 3,4-dihydroxy-α-methylcinnamic acid amide of neo-inosamine-2. The latter is isomeric, and possibly identical with, the corresponding degradation product of hygromycin (2). Structure of the degradation product:



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Hygroscopins

Produced by: Streptomyces hygroscopicus (1, 2).

Synonyms: Hygroscopin A and elaiomycin are similar or closely related.

Method of extraction: Broth extracted with butyl acetate at pH 2.0. Extract washed with sodium bicarbonate solution, then concentrated *in vacuo*. Concentrate extracted with methanol. Extract chromatographed on carbon to give three fractions. Fraction I (eluted with methanol) gives hygroscopin B on distillation. Fraction II (eluant, methanol) contains hygroscopins A and B, which are separated by distillation or carbon chromatography. Fraction III (eluant, ethyl acetate) gives hygroscopin C (1, 3).

Chemical and physical properties: Hygroscopin A: Oil; b.p.$_{0.003}$ 64°C. $[\alpha]_D^{14} = +84.7°$ (methanol). Ultraviolet absorption spectrum maximum at 235 mμ (ethanol). Refractive index: n_D^{13} 1.4830. $C_{13}H_{24}O_3N_2$. *Hygroscopin B:* Oil; b.p.$_{0.008}$ 70°C or b.p.$_{0.5}$ 105°C. $[\alpha]_D^{14} = -38.8°$ (methanol). Ultraviolet absorption spectrum maximum at 233 mμ (ethanol). Refractive index: n_D^{14} 1.4935. Infrared spectrum given in reference 1. $C_{15}H_{28}O_3N_2$: C = 63.53%; H = 10.05%; N = 10.07%. Molecular weight, 290 ± 10. *Hygroscopin C:* Not characterized (1, 3).

Biological activity: Hygroscopin A: Active on fungi, yeasts, influenza A virus (in tissue culture), and *M. tuberculosis* var. *hominis* H37Rv. Transiently active on Yoshida sarcoma (rats) at 1 mg per kg. *Hygroscopin B:* Active on influenza A virus (as above) but not on fungi, yeasts, or mycobacteria. Same activity as hygroscopin A on Yoshida sarcoma.

Toxicity: Hygroscopin A: LD_{50} (mice) 8.75 mg per kg intraperitoneally. *Hygroscopin B:* LD_{50} (mice) 1928 mg per kg intraperitoneally.

References:

1. Tatsuoka, S. *et al.* J. Antibiotics (Japan) **7B:** 329–332, 1954.
2. Nakazawa, K. *et al.* J. Agr. Chem. Soc. Japan **28:** 296–299, 1954.
3. Tatsuoka, S. *et al.* J. Antibiotics (Japan) **8A:** 31, 1955.

Hygrostatin

Produced by: Streptomyces hygrostaticus.

Synonym: Similar to musarin.

Method of extraction: Mycelium collected and washed with water. Extraction from mycelium with methanol or acetone. Solvent concentrated. Concentrate extracted with butanol at pH 6.5. Precipitation from butanol with ether. Precipitate dissolved in a 1:1 mixture of benzene and methanol, and chromatographed over alumina. Elution carried out by increasing the methanol content of the mixture. Further purification by countercurrent distribution in a chloroform-methanol-pH 7.0 phosphate buffer system (1:1:0.5). Active fractions evaporated and dissolved in butanol. Upon addition of ether, a precipitate forms, which then is dried.

Chemical and physical properties: Pale yellow powder. Decomposes 129–131°C. Contains N but no S or halogens. Very soluble in methanol and pyridine. Soluble in ethanol, butanol, and isopropanol. Slightly soluble in benzene, chloroform, and acetone. Insoluble in ethyl acetate, ether, petroleum ether, dioxane, and water. Dark red to purple color with concentrated sulfuric acid. Orange-brown to red-purple with concentrated HCl. Negative biuret, $FeCl_3$, Benedict, ninhydrin, Fehling, and Tollen tests. Acid hydrolysate also ninhydrin-negative. $[\alpha]_D^{20} = +43°$ (c = 1.21 per cent in methanol). Light absorption maximum at 240 mμ ($E_{1cm}^{1\%}$ 360) and a shoulder at 255 to 270 mμ. Stable for 30 minutes at 100°C at neutrality. Unstable at acid and alkaline pH values.

Biological activity: Active against filamentous fungi, yeasts, and gram-positive bacteria. Inactive against gram-negative bacteria and clostridia

Toxicity: LD_{50} (mice) 8.6 mg per kg intravenously, 21.7 mg per kg intraperitoneally, 246.7 mg per kg subcutaneously, and 530 mg per kg orally. Necrosis at the site of injection. Autopsy shows bleeding in the lungs. Nontoxic at 500 μg per ml when sprayed on plant leaves.

Reference: 1. Kojo, K. *et al.* Yakugaku Kenkyu **30:** 654–664, 1958.

Isorhodomycins

Produced by: Streptomyces purpurascens (1). This culture also produces rhodomycins A and B.

Remarks: See rhodomycins.

Method of extraction: See rhodomycin A. Crude isorhodomycin A from paper chromatography taken up in a small amount of ethanol. A drop of concentrated HCl added. Cooling gives crystals (2).

Chemical and physical properties: Isorhodomycin A: Dark red prisms; m.p. 220°C. Soluble in water and low-molecular weight alcohols. Very slightly soluble in benzene and chloroform. Insoluble in ether and petroleum ether. Red fluorescence under ultraviolet light. Ultraviolet light-absorption spectrum maxima at about 235, 305, 525, 551, 563, and 610 mμ (methanol). $[\alpha]^{18}_{606\text{-}760} = +268° \pm 30°$ (c = 0.1 per cent in methanol). $C_{20\text{-}21}H_{29\text{-}31}O_8N\cdot HCl$: C = 54.25%; H = 6.84%; O = 27.92%; N = 3.12%; Cl = 7.3%. *Perchlorate:* Thin red needles; m.p. 177°C (2). *Isorhodomycin B:* Crimson-red substance (3).

Biological activity: Active on *Staph. aureus* (2).

References:

1. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
2. Brockmann, H. and Patt, P. Chem. Ber. **88:** 1455–1468, 1955.

Kanamycin A

Produced by: Streptomyces kanamyceticus. Broths contain a second, butanol-soluble substance, active on *B. subtilis* (2), as well as kanamycin B (see next abstract). Rf values for the *B. subtilis* factor, kanamycin A and B, respectively, are 0, 0.1 to 0.26, and 0.21 to 0.37 on paper chromatography (2 per cent *p*-toluene sulfonic acid) (9).

Method of extraction: I. Adsorbed from broth-filtrates on IRC-50 (Na^+ form), and eluted with HCl. Eluate neutralized, diluted, and resorbed on IRC-50 (regenerated with NH_4OH). Eluted with 0.2 *N* NH_4OH; eluate concentrated *in vacuo*, diluted with methanol, and adjusted to pH 8.0 to 8.2 to precipitate kanamycin sulfate. Repeated recrystallization from methanol-water at pH 7.8 to 8.2. Converted to the base by treatment of an aqueous solution of the sulfate with a strongly basic ion exchange resin, concentration, and crystallization with methanol-ethanol (6). II. Broths treated with IRC-50 and eluted as in I. Eluates adjusted to pH 6.0 to 8.0, evaporated *in vacuo*, and lyophilized. Powder taken up in methanol, filtered, and acetone added to give a precipitate. Precipitated as the reineckate, then converted to other salts (2, 3).

Chemical and physical properties: Tetraacidic base. *Base:* Soluble in water. Insoluble in non-polar organic solvents (10). $[\alpha]^{24}_D = +146°$ (c = 1 per cent in 0.1 *N* H_2SO_4). Positive ninhydrin, Molisch, and Elson-Morgan tests (6). The two latter tests were at first mistakenly reported as being negative (2). Blue-violet color with the biuret test (24). Negative reducing sugar, Tollen, Sakaguchi, and maltol tests (6). Stable; can be autoclaved for 1 hour at 120°C in aqueous solution with only 10 per cent loss of activity (10). Acid hydrolysis products include 2-desoxystreptamine (1,3-diamino-4,5,6-trihydroxycyclohexane, which is also obtained from neamine) and two amino sugars: 6-amino-6-desoxy-D-glucose and 3-amino-3-desoxy-D-glucose (also known as kanosamine). C = 44.8%; H = 7.5%; N = 11.3%. $C_{18}H_{36}N_4O_{11}$. Structural formula (6, 7, 17) of kanamycin A given in Chapter 6. *Sulfate:* White, irregular, prismatic crystals (hydrate) (6) or plates (3). No melting point; decomposes over a wide range above 250°C (6). Soluble in water but insoluble in organic solvents (2). $[\alpha]^{23}_D = +121°$ (c = 1 per cent in H_2O) (3). *Hydrochloride:* Hygroscopic, white, amorphous powder. Very soluble in water, soluble in methanol, and slightly soluble in ethanol. Insoluble in acetone, ethyl acetate, butyl acetate, ether, benzene, and petroleum ether. No characteristic absorption maxima in ultraviolet light (2). Infrared spectrum given in reference 2. $[\alpha]^{20}_D = +103°$ (c = 1 per cent in H_2O). More stable at pH 6 to 8 than pH 2.0 (2). *Reineckate:* Darkens at 191–193°C, decomposes at 211–213°C (24). *Picrate:* Crystalline; m.p. 225–230°C (decomposition) (6). *Tetra-N-acetyl kanamycin:* Crystalline; m.p. 250–255°C (decomposition) (6). Characteristics of a variety of Schiff bases formed by kanamycin given in reference 6.

Biological activity: Active on gram-positive and gram-negative bacteria, including actinomycetes and mycobacteria. Relatively inactive on streptococci, diplococci, clostridia, and *Pseudomonas.* Not active on fungi (2, 18). Most active at alkaline pH (18). Active *in vivo* (mice and guinea pigs) on *M. tuberculosis* H37Rv, but is less effective than isoniazid and slightly less so than streptomycin. No cross-resistance with *p*-aminosalicylic acid, cycloserine, streptovaricin, streptomycin, or isoniazid. Partial cross-resistance with phleomycin when *E. coli* is used as the test organism, but not when *Mycobacterium* 607 is used. Cross-resistance with streptothricin, neomycin, and viomycin *in vitro.* Kanamycin causes fragility of

the cell wall in *E. coli*. Resistance to *E. coli* develops slowly, in stepwise fashion. Resistance of mycobacteria develops rapidly (3, 4, 9, 18). Active in mice on *D. pneumoniae*, *Sal. typhosa*, *Staph. aureus*, and *Pr. vulgaris* infections. Not active on *Streptococcus hemolyticus* (1, 2, 11). Preventive action on mouse leprosy, leptospira in guinea pigs, and *Treponema pallidum* infections in rabbits (8, 9). Not active on sarcoma 180 or Ehrlich carcinoma (16).

Toxicity: Crystalline sulfate: LD_{50} (rats) 415 to 830 mg per kg intravenously; (rabbits) 225 to 300 mg per kg intravenously (3). LD_{50} (mice) 167.9 mg per kg intraperitoneally, 1648 mg per kg subcutaneously, and 316.3 mg per kg intravenously. Doses greater than 10,000 mg per kg are tolerated orally (poorly absorbed from the intestinal tract). Less vestibular toxicity to cats than streptomycin; less auditory toxicity to rats than dihydrostreptomycin. Much less nephrotoxicity to dogs than neomycin (5). Nephrotoxic and ototoxic in human beings, but does not have the delayed ototoxicity of neomycin (20).

Utilization: Active on certain diseases caused by gram-positive and gram-negative organisms. Can be given orally, parenterally, or topically (19). Not considered effective in infections caused by enterococci, pneumococci, or anaerobes (20). Somewhat active on tuberculosis (21). Relief of typhoid and *Endamoeba histolytica* carrier states (22, 23) and can be used safely in patients allergic to streptomycin (12). Possibly beneficial in cirrhosis (13). Bowel sterilization (14). Bacterial infections resistant to commonly used antibiotics (15).

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2. Umezawa, H. *et al.* J. Antibiotics (Japan) **10A:** 181–188, 1957.
3. Maeda, K. *et al.* J. Antibiotics (Japan) **10A:** 228–231, 1957.
4. Steenken, W., Jr. *et al.* Trans. 17th Veterans Admin. Conf. Chemotherapy Tuberc. 386–391, 1958.
5. Dickison, H. L. and Tisch, D. E. Trans. 17th Veterans Admin. Conf. Chemotherapy Tuberc. 391–397, 1958.
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10. Cron, M. J. *et al.* Ann. N. Y. Acad. Sci. **76:** 27–30, 1958.
11. Hunt, G. A. and Moses, A. J. Ann. N. Y. Acad. Sci. **76:** 81–87, 1958.
12. Donomae, I. Ann. N. Y. Acad. Sci. **76:** 166–187, 1958.
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15. Yow, E. M. and Monzon, O. T. Ann. N. Y. Acad. Sci. **76:** 372–390, 1958.
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17. Umezawa, S. *et al.* J. Antibiotics (Japan) **11A:** 162, 1958.
18. Morikubo, Y. J. Antibiotics (Japan) **11A:** 171–180, 1958.
19. Russo, J. J. and Mountain, C. Antibiotics Ann. 605, 1958–1959.
20. Finegold, S. M. *et al.* Antibiotics Ann. 606–622, 1958–1959.
21. Shapiro, M. and Hyde, L. Antibiotics Ann. 708–710, 1958–1959.
22. Bettag, O. L. *et al.* Antibiotics Ann. 721–724, 1958–1959.
23. Ruiz Sanchez, F. R. *et al.* Antibiotics Ann. 725–735, 1958–1959.
24. Japanese Patent 3749, May 18, 1959.

Kanamycin B

Produced by: Streptomyces kanamyceticus. This culture also produces kanamycin A and another antibiotic (see kanamycin A).

Method of extraction: Isolated by countercurrent distribution of salicylidene derivatives of the mixture of kanamycins A and B (methanol-water-chloroform-benzene, 5:4:2:1) and chromatography on Amberlite XE-64 (NH_4^+ form) with 0.08 *N* NH_4OH. Recrystallized from 90 per cent ethanol (1).

Chemical and physical properties: Basic substance. Decomposes over a wide range above 170°C. Soluble in water; slightly soluble in lower alcohols; insoluble in nonpolar organic solvents. Infrared data given in reference 1; spectrum is said to resemble kanamycin A. $[\alpha]_D^{24} = +135°$ (c = 0.63 per cent in H_2O). Positive Molisch, Elson-Morgan, and ninhydrin tests. Negative Fehling and Benedict tests. C = 44.75%; H = 7.50%; N = 12.55%. Yields Schiff bases with aromatic aldehydes. Unlike kanamycin A, yields no furfural after sulfuric acid treatment. Acid hydrolysates contain desoxystreptamine, kanosamine, and one unidentified ninhydrin-positive spot,

but no 6-glucosamine (6-desoxy-6-amino-D-glucose) as with kanamycin A. *N-acetyl kanamycin B:* Decomposes gradually at 220–250°C. $[\alpha]_D^{24} = +150°$ (c = 0.42 per cent in water, 1).

Biological activity: Active on gram-positive and gram-negative bacteria, including mycobacteria. Almost 2 to 3 times as active as kanamycin A against bacteria such as *Aerobacter aerogenes, B. cereus, B. subtilis, Br. bronchiseptica, E. coli, K. pneumoniae, Staph. aureus,* and *Proteus* spp. Not active on clostridia or *C. albicans, Salmonella,* or *Serratia marcescens.* Neither kanamycin A nor B is very active on streptococci. B is less active than A on mycobacteria (2).

Toxicity: More toxic to animals than kanamycin A (3).

References:

1. Schmitz, H. *et al.* J. Am. Chem. Soc. **80:** 2911–2912, 1958.
2. Gourevitch, A. *et al.* Antibiotics & Chemotherapy **8:** 149–159, 1958.
3. Hubef, K. Quoted in Finegold, S. N. *et al.* Antibiotics Ann. 606–622, 1958–1959.

Lagosin

Produced by: Streptomyces sp. (1).

Synonym: Antibiotic A 246.

Method of extraction: Mycelium (60 per cent of the activity) extracted with 80 per cent aqueous acetone. Extract concentrated *in vacuo.* A small amount of n-butanol added to the aqueous residue and the water removed by concentration *in vacuo.* Precipitated from residual solution by addition of diethyl ether in excess. Filtrate (40 per cent of the activity) extracted with n-butanol. Extracts concentrated, then treated as above (1).

Chemical and physical properties: Macrocyclic lactone, with pentaene chromophore. Crystalline substance; m.p. about 235°C (decomposition). $[\alpha]_D^{20} = -160°$ (c = 0.2 per cent in methanol). Ultraviolet absorption spectrum maxima at 325, 340, and 358 mμ ($E_{1cm}^{1\%}$ 1491). $C_{41}H_{66\text{-}70}O_{14}$. Perhydro derivative: $C_{41}H_{78\text{-}80}O_{14}$; m.p. 156–157°C. $[\alpha]_D^{20} = +3.5°$ (c = 1.98 per cent in methanol) (1–3). Structural formula given in Chapter 6 and reference 4.

Biological activity: Active on yeasts and filamentous fungi (1).

References:

1. Ball, S. *et al.* J. Gen. Microbiol. **17:** 96–103, 1957.
2. Dhar, M. L. *et al.* Proc. Chem. Soc. 148–149, 1958.
3. Dhar, M. L. *et al.* Proc. Chem. Soc. 154–155, 1958.
4. Dhar, M. L. *et al.* Proc. Chem. Soc. 310–311, 1960.

Lavendulin

Produced by: Streptomyces lavendulae (1).

Synonym: Streptothricin-like antibiotic.

Method of extraction: Like that for actinorubin.

Chemical and physical properties: Basic substance. *HCl salt:* White powder. *Helianthate:* Orange needles in clusters; m.p. 212–220°C (decomposition). Soluble in 80 per cent aqueous methanol; insoluble in 20 per cent methanol. C = 51.16%; H = 5.99%; N = 17.32%; S = 9.17%. Probable empirical formula: $C_{49}H_{63}O_{18}N_{13}S_3$ (1). Major component in broth indistinguishable from streptothricin on paper chromatography (wet butanol-*p*-toluenesulfonic acid) (4).

Biological activity: Active on gram-positive and gram-negative bacteria, including mycobacteria. Slightly active on *Trichophyton interdigitale* (16 μg per ml). Cross-resistance with actinorubin and streptothricin (3).

Toxicity: LD_{100} (mice) 28.5 mg per kg intraperitoneally. Toxic effects at therapeutic levels (2).

References:

1. Junowicz-Kocholaty, R. and Kocholaty, W. J. Biol. Chem. **168:** 757–764, 1947.
2. Morton, H. E. Proc. Soc. Exptl. Biol. Med. **64:** 327–331, 1947.
3. Kelner, A. and Morton, H. E. J. Bacteriol. **53:** 695–704, 1947.
4. Benedict, R. G. Botan. Rev. **19:** 229–320, 1953.

Lenamycin

Produced by: Streptomyces sp.

Method of extraction: Broth adjusted to pH 4.0, filtered, then extracted with n-butyl acetate. The aqueous phase concentrated *in vacuo* and the gummy brown substance which precipitates on addition of absolute ethanol is discarded. Aqueous ethanolic filtrate treated with alumina, then concentrated *in vacuo* with addition of absolute methanol. Addition of acetone gives an inactive white precipitate. Concentrate of the mother liquor gives a crude precipitate of lenamycin on standing in desiccator. Recrystallization from methanol.

Chemical and physical properties: Organic acid amide. Needles; m.p. 202–207°C (decomposition) (d.p. 290–300°). Optically inactive. C = 40.89%; H = 4.42%; N = 22.91%. No S, halogen, or metals. $C_4H_4N_2O_{2\text{-}3}$. Ultraviolet absorption spectrum maximum at 216 mμ ($E_{1cm}^{1\%}$ 817). Infrared absorp-

tion spectrum given in reference 1. Negative ninhydrin, biuret, anthrone, $FeCl_3$, Elson-Morgan, and Sakaguchi tests. No nitro or oxime groups. Unstable to alkaline pH; more stable at acid pH.

Biological activity: Active on HeLa cells in tissue culture (human cervical carcinoma cell).

Reference: 1. Sekizawa, Y. J. Biochem. (Tokyo) **45:** 159–162, 1958.

Leucomycin

Produced by: Streptomyces kitasatoensis strains (2, 9) and *Streptomyces* sp. (12).

Synonym: Antibiotic C 637 (12).

Method of extraction: I. Culture medium acidified, stirred with "Dicarite," and filtered. Filtrate extracted with benzene at pH 7.2 to 9.0. Back-extracted into water at pH 3 to 4. Extracted into ether at pH 9.0. Extract evaporated to dryness. Purified by treating a methanolic solution with activated carbon, then chromatographing on alumina from benzene, developed with acetone-benzene (3:7). Precipitated as the tartrate. Broth contains three components; mycelium, a fourth. The three bases are demonstrable on countercurrent distribution (isopropyl ether-0.062 *M* phosphate buffer pH 6.25, 1:1) (5).

Chemical and physical properties: Basic substances. *Complex:* Yellowish rhomboid crystals; m.p. 126–129°C (1, 5). Soluble in alcohols, acetone, ethyl acetate, butyl acetate, chloroform, ether, and benzene. Slightly soluble in water. Insoluble in petroleum ether. Ultraviolet absorption spectrum maximum at 230 to 232 mμ ($E^{1\%}_{1\,cm}$ 228) and a weak band at 285 mμ ($E^{1\%}_{1\,cm}$ 8.6) (ethanol) (4). Infrared absorption spectrum given in reference 1. $[\alpha]^{25}_{D} = -60.42°$ (c = 2 per cent in ethanol). pK_b = 7.5 (water). Positive Molisch, Schiff, Tollen, and Seliwanoff tests. Negative Fehling, Benedict, biuret, ninhydrin, Sakaguchi, glucosamine, xanthoproteic, $FeCl_3$, and maltol tests. Violet color in concentrated HCl, brown-purple in concentrated H_2SO_4, and yellow in alkaline methanol. Gives a precipitate with trichloroacetic acid (1, 4). $C_{33-38}H_{54-66}NO_{11-13}$: C = 61.32%; H = 8.61%; N = 2.03%. *Hydrochloride:* White, column-shaped crystals. *Acetate:* Crystalline substance; m.p. 135.5°C. Biologically active. *Tartrate:* White needles; m.p. 125°C. Very soluble in water, alcohols, and acetone. Slightly soluble in chloroform and ether. Insoluble in benzene, petroleum ether, dichloroethylene, and trichloroethylene. Aqueous solution loses 28 per cent of its potency at room temperature in 15 days (1). *Base 0:* m.p. 135°C. C = 60.1%; H = 8.4%; O = 28.0%; N = 1.6%. pK_b = 7.2. Ultraviolet spectrum maxima at 231 mμ ($E^{1\%}_{1\,cm}$ 333) and 279 mμ ($E^{1\%}_{1\,cm}$ 5). Rf = 0.03 (stationary phase: disodium phosphate; mobile phase: cyclohexane-methyl isobutyl ketone, 85:15). *Base I:* m.p. 142–143°C. $[\alpha]^{20}_{D} = -76°$ (c = 1 per cent in methanol). C = 60.1%; H = 8.6%; O = 28.5%; N = 1.7%. pK_b = 7.1. Ultraviolet absorption spectrum maxima at 231 mμ ($E^{1\%}_{1\,cm}$ 296) and 279 mμ ($E^{1\%}_{1\,cm}$ 2.5). Rf = 0.03 (above system). *Base II:* m.p. 139°C. C = 60.2%; H = 8.3%; O = 29.1%; N = 1.5%. Ultraviolet absorption spectrum maxima at 231 mμ ($E^{1\%}_{1\,cm}$ 330) and 279 mμ ($E^{1\%}_{1\,cm}$ 2.2). Rf = 0.15 (above system) (8).

Biological activity: Active on gram-positive bacteria and mycobacteria. Not active on gram-negative bacteria, except the *Hemophilus-Neisseria* group. Not active on filamentous fungi or yeasts (1). Cross-resistance with oleandomycin but not erythromycin (11, 12). Less active at alkaline than at acid pH. Active *in vivo* against *Staph. aureus, Streptococcus pyogenes, Clostridium welchii* (guinea pig), *Borrelia duttonii* (mice), *Rickettsia tsutsugamushi* (mice), *Rickettsia prowazekii* (chick embryos), lymphogranuloma (mice), and sheep infectious pneumonia. Moderately active on *Treponema pallidum* (guinea pigs and rabbits). Not active on *Trypanosoma evansi* (mice) or rabies virus (mice) (3, 7, 12). Addition to the soil in which a cucumber seedling is growing doubles the curvature caused by a one-sided growth of the hypocotyl that occurs when one of the cotyledons is shaded (6).

Toxicity: LD_{50} (mice) 650 mg per kg intravenously, >800 mg per kg subcutaneously. Mice tolerate >2 gm per kg of the tartrate orally (1).

Utilization: Effective in a case of chronic cholecystitis (10).

References:

1. Hata, T. *et al.* J. Antibiotics (Japan) **6A:** 87–89, 1953.
2. Hata, T. *et al.* J. Antibiotics (Japan) **6A:** 109–112, 1953.
3. Hata, T. *et al.* J. Antibiotics (Japan) **6A:** 163–171, 1953.
4. Sano, Y. *et al.* J. Antibiotics (Japan) **7A:** 88–92, 1954.
5. Sano, Y. J. Antibiotics (Japan) **7A:** 93–97, 1954.
6. Kribben, F. J. Naturwissenschaften **41:** 144–145, 1954.
7. Hashimoto, T. *et al.* Japan. J. Bacteriol. **10:** 787–790, 1955.
8. Despois, R. *et al.* Giorn. microbiol. **2:** 76–90, 1956.
9. Nakamura, G. *et al.* J. Antibiotics (Japan) **9B:** 213–217, 1956.

10. Yoshida, R. and Tsuruma, M. J. Antibiotics (Japan) **10A:** 177, 1957.
11. Welch, H. *et al.* Antibiotics Ann. 337–341, 1958–1959.
12. Steinberg, B. A. *et al.* Antibiotics Ann. 342–345, 1958–1959.

Leucomycin B

Produced by: Streptomyces kitasatoensis (1).

Remarks: Produced in broths coincidentally with leucomycin.

Method of extraction: Filtered broth extracted with benzene or butyl acetate at pH 8.0. Back-extracted into dilute HCl (pH 4.0), washed with butyl acetate, and adjusted to pH 8.0 with 1 N NaOH. Alkaline solution extracted with benzene. After concentration of the extract to a small volume, carbon is added. Elution from carbon with hot benzene. Benzene solution concentrated *in vacuo*. Leucomycin B precipitated in the cold. Recrystallized from aqueous ethanol. Differentiated from leucomycin by paper chromatography (ether) (1).

Chemical and physical properties: White needles; m.p. 192–193°C. Basic compound. Relatively insoluble in water. Soluble in ether, acetone, chloroform, ethyl acetate, butyl acetate, and benzene. Acid salts are water-soluble. $[\alpha]_D^{20} = -49.6°$ (c = 2 per cent in ethanol). $C_{41}H_{69}NO_{16}$: C = 59.34%; H = 8.31%; N = 1.65%; O = 30.71%, by difference. Ultraviolet absorption spectrum maximum at 232 mμ ($E_{1\,cm}^{1\%}$ 325). Infrared absorption spectrum given in reference 1. Biologically active derivatives include acetyl leucomycin B (I), the thiosemicarbazone (II), 2,4-dinitrophenylhydrazone (III), and isonicotinic hydrazone (IV) (1).

Biological activity: Leucomycin B is active on gram-positive bacteria and relatively inactive on mycobacteria, gram-negative bacteria, fungi, *Candida*, and *Nocardia asteroides*. Derivatives I, II, III, and IV are active on gram-positive bacteria and mycobacteria.

Reference: 1. Sano, Y. J. Antibiotics (Japan) **9:** 202–206, 1956.

Leucomycin-like Complex

Produced by: Streptomyces sp. with some similarities to *S. kitasatoensis*.

Synonym: Antibiotic 6, 237 R.P.

Method of extraction: Broth-filtrate extracted with amyl acetate at pH 8. Extract treated with acidic water. Readjustment to alkaline pH and extraction with ethylene dichloride. Evaporation gives crude substance. Purification by countercurrent distribution (isopropyl ether-0.062 *M* phosphate buffer pH 6.5, 1:1) and chromatography on alumina from benzene.

Chemical and physical properties: Complex substance composed of two bases. *Base I:* m.p. 139–140°C. $[\alpha]_D^{20} = -78°$ (c = 1 per cent in methanol). C = 60.5%; H = 8.5%; O = 27.8%; N = 1.85%. $pK_b = 7.1$. Ultraviolet absorption spectrum maxima at 231 mμ ($E_{1\,cm}^{1\%}$ 311) and 279 mμ ($E_{1\,cm}^{1\%}$ 6.3). Rf = 0.03 (stationary phase: disodium phosphate; mobile phase: cyclohexane-methyl isobutyl ketone, 85:15). *Base II:* m.p. 144°C. $[\alpha]_D^{20} = -75°$ (c = 1 per cent in methanol). C = 60.5%; H = 8.4%; O = 29.3%; N = 1.6%. $pK_b = 7.1$. Ultraviolet absorption spectrum maxima at 231 mμ ($E_{1\,cm}^{1\%}$ 348) and 279 mμ ($E_{1\,cm}^{1\%}$ 2.3). Rf = 0.15 (system given above). Leucomycin contains more Base I than Base II. The reverse is true in this complex. A third base, present in the original leucomycin, is not present in this complex.

Biological activity: Active on gram-positive bacteria. Not active on gram-negative bacteria, except *Neisseria*, *Pasteurella*, etc. Cross-resistance with erythromycin and carbomycin. Active in mice on streptococcal, pneumococcal, and staphylococcal infections.

Toxicity: LD_{50} (mice) about 2 gm per kg subcutaneously, >5 gm per kg orally.

Reference: 1. Despois, R. *et al.* Giorn. microbiol. **2:** 76–90, 1956.

Levomycin

Produced by: Streptomyces sp.

Synonyms: Similar to actinoleukin and antibiotic F 43.

Method of extraction: Extracted from broth at all pH values by ethyl acetate, n-butyl alcohol, or ether. A pigmented impurity can be removed by treatment of the concentrated ethyl acetate extract in the cold with 0.01 *N* NaOH. Precipitated from washed and concentrated ethyl acetate solutions by petroleum ether or n-hexane. Purification by countercurrent distribution (methanol-benzene-water, 5:5:1). Crystallization of most active fraction from cold chloroform-ethanol. Chromatography over silicic acid in chloroform-methanol.

Chemical and physical properties: Colorless prisms; m.p. 222–224°C. $C_{27}H_{38}N_6O_{10}$: C = 54.05%; N = 13.90%; H = 6.35%. Very soluble in chloroform and pyridine; less so in carbon tetrachloride, ethyl acetate, and hot alcohols. Slightly soluble in ether, benzene, cold alcohols, acetone, and dioxane. Insoluble in water, petroleum ether, 5 per cent aqueous HCl, and NaOH. Soluble in cold 6 *N* HCl with slow decomposition. $[\alpha]_D^{25} =$

$-290°$ (c = 2 per cent in acetone). Ultraviolet absorption maxima at 318 mμ ($E^{1\%}_{1\,cm}$ 185) and 243 mμ ($E^{1\%}_{1\,cm}$ 1200). Infrared absorption spectrum given in reference 1. Saponification equivalent, 427 to 489. Negative ninhydrin, biuret, Millon, Hopkins-Cole, Pauly, Sakaguchi, maltol, Benedict, Tollen, 2,4-dinitrophenylhydrazine, bromine water, bromine (CCl_4), $KMnO_4$, periodate, methanolic $FeCl_3$, Molisch, zinc-ammonium chloride, Zeisel alkoxyl, and hydroxamic acid tests. Positive pine-splint test. Treatment with cold methanolic NaOH yields inactive levomycic acid (m.p. 155–160°C) with an infrared spectrum similar to levomycin. Vigorous alkaline and acid hydrolyses reveal presence of at least four ninhydrin-positive components, a yellow fluorescent pigment, and a volatile acid. Possibly a peptide with a chromophoric moiety.

Biological activity: Moderately active on gram-positive and gram-negative bacteria and mycobacteria.

Toxicity: LD_{100} (mice) 44 mg per kg intravenously.

Reference: 1. Carter, H. E. *et al.* Arch. Biochem. Biophys. **53:** 282–293, 1954.

Litmocidin

Produced by: Nocardia cyanea (*Proactinomyces cyaneus-antibioticus*) (1).

Method of extraction: Water-extract of agar culture acidified to pH 3.5 and treated with charcoal. Elution with acidic acetone. Eluate evaporated to dryness *in vacuo*. Residue taken up in ethanol, then precipitated by addition of water. To an acidified (HCl) solution, ether, then water, is added until an aqueous phase separates, leaving the antibiotic in the ether layer. Reprecipitated from ethanol to give red form. Red form dissolved in ethanol, neutralized, and ethanol evaporated *in vacuo* to give the blue form (2).

Chemical and physical properties: Possibly related to anthocyanin pigments, but differing from them in many respects. Red at acid pH, violet at neutrality, and blue at alkaline pH. *Red form:* m.p. 144–146°C. Slightly soluble in acidic water. Soluble in ethanol, acetone, and alkaline water. Ultraviolet absorption spectrum maxima at 460 to 480 mμ, 510 to 530 mμ, and 560 to 570 mμ. Dry powder stable. Aqueous solutions more stable at acid than alkaline pH. Positive $FeCl_3$ test. Blue precipitate with lead acetate. Decolorized by zinc dust; color restored on exposure to air. Decolorized by bisulfite; color not restored with strong acid. Does not form salts with mineral acids. Strong alkali at room temperature destroys the biological activity but not the color. Heating with 20 per cent HCl for 10 hours at 80–90°C affects the color, but not the activity. No carbohydrate present. On alkaline hydrolysis gives two fractions: an acid fraction giving a positive $FeCl_3$ test, and an alkaline fraction which does not contain phloroglucinol. *Blue form:* Very soluble in water. *Picrate:* Red substance; m.p. 100–102°C (2, 3).

Biological activity: Active on gram-positive bacteria and mycobacteria. Very slightly active on gram-negative bacteria. Activity unaffected by horse serum. Not active on *Staph. aureus* in mice (1).

Toxicity: LD_{50} (mice) about 50 mg per kg intraperitoneally (1).

References:

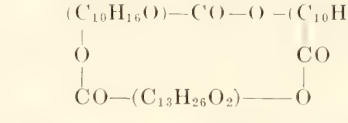
1. Gause, G. F. J. Bacteriol. **51:** 649–653, 1946.
2. Brazhnikova, M. G. J. Bacteriol. **51:** 655–657, 1946.
3. Paskhina, T. S. Biokhimiya (Engl. transl.) **21:** 453–455, 1956.

Longisporin

Produced by: Actinomyces (*Streptomyces*) *longisporus* (1).

Method of extraction: Broth and mycelium extracted with chloroform. Mycelium re-extracted four times with chloroform. Extracts evaporated first at atmospheric pressure, then *in vacuo*. Residue taken up in anhydrous ether, and treated by passing over an alumina column. Ether taken to dryness. Residue taken up in absolute alcohol or petroleum ether (b.p. 60–80°C). Crystallization occurs slowly in the cold. Recrystallized from absolute alcohol, then petroleum ether (1).

Chemical and physical properties: Neutral substance. Large snow-white prisms; m.p. 99–101°C. Soluble in chloroform, ethanol, and petroleum ether. Insoluble in water, acid, or alkali. $[\alpha]_D$ = +2.62° (c = 5 per cent in chloroform). C = 66.44%; H = 9.03%; No N, S, or P. Molecular weight, 651.2. $C_{36}H_{58}O_{10}$. Product of degradation with alcoholic KOH is an acid, $C_{11}H_{18}O_4$; m.p. 64–65°C. Preliminary structural formula of longisporin (1):



Biological activity: Active *in vitro* against mycobacteria. Active on other gram-positive bacteria (1).

Toxicity: Too toxic for clinical use. Hemolytic (1).

Reference: 1. Men'shikov, G. P. and Rubinstein, M. M. Zhur. Obshcheĭ Khim. **26:** 2035–2039, 1956.

Luridin

Produced by: Streptomyces luridus (1).

Method of extraction: Adsorption on charcoal at pH 6.0 to 6.5. Elution with aqueous methanol at pH 2.0 to 2.5. Precipitation with acetone. Further purification by formation of picrate, which is transformed into a hydrochloride (2).

Chemical and physical properties: Basic substance. Negative maltol and ninhydrin tests. Positive Pauly and biuret tests. Faint Molisch reaction (2).

Biological activity: Same general activity as antibiotics of the streptothricin group. Active against the virus of silkworm jaundice (2). Culture filtrates active *in ovo* against influenza A virus (1).

Toxicity: Toxicity in animals is of the streptothricin type (2).

References:

1. Krassilnikov, N. A. *et al.* Mikrobiologiya **26:** 558, 1957.
2. Trakhtenberg, D. M. *et al.* Antibiotiki **4(2):** 9–13, 1959.

Lustericin

Produced by: Streptomyces sp.

Method of extraction: Broth extracted with ethyl acetate; mycelium with methanol. Extracts concentrated *in vacuo* at low temperature. Acetone is added to the residues, and the whole filtered. Acetone removed from the filtrate by vacuum distillation, and the residue extracted with ethyl acetate. Chromatographed on Magnesol-Dicalite with acetone as solvent and developer. Active fractions concentrated *in vacuo* and crystallized from ethanol. Recrystallized from hot ethanol.

Chemical and physical properties: Thin colorless needles; m.p. 130°C. Soluble in methanol, ethanol, acetone, ethyl acetate, and chloroform. Sparingly soluble in petroleum ether. Insoluble in water. End-absorption in ultraviolet light. Infrared spectrum given in reference 1. Optically inactive in methanol. $C_{40}H_{64}O_{13}$: C = 63.87%; H = 8.71%. Molecular weight, 780 ± 60.

Biological activity: Active on gram-positive bacteria at 2 to 5 μg per ml and on mycobacteria at 20 to 50 μg per ml. Active on *C. albicans* and *P. notatum* at 5 μg per ml and *Sacch. cerevisiae* and *A. niger* at 50 μg per ml.

Toxicity: LD_{50} (mice) 125 to 150 mg per kg intraperitoneally.

Reference: 1. Shibata, M. *et al.* Ann. Rept. Takeda Research Lab. **17:** 19–22, 1958.

Luteomycin

Produced by: Streptomyces tanashiensis (resembling *S. aureus*) (1, 6, 9); *Streptomyces* sp. (resembling *S. tanashiensis*) (3, 14); *S. flaveolus* (16); *Streptomyces* sp. (4, 5).

Synonyms: Antibiotic H 2053 (12). Probably: Special substance 3 (K 349) (3) and substance 1 (14).

Method of extraction: Broth-filtrate extracted with ethyl or butyl acetate, chloroform, or ether at pH 7.5 to 8.0. Back-extracted into water at pH <3.0. Chromatographed on alumina from a water-insoluble solvent and developed with a water-soluble solvent. Crystallization occurs as the active fractions are dehydrated (6), or by addition of ether to an acetone solution (2).

Chemical and physical properties: Basic substance. *Free base:* Very soluble in benzene. Soluble in water and common organic solvents. Ultraviolet absorption spectrum maxima at 280 and 420 to 440 mμ (0.1 *N* HCl) or 270 to 280 and 500 mμ (0.1 *N* NaOH) (6). *Hydrochloride:* Orange-yellow rhomboid or needle crystals; m.p. 199–200°C (decomposition). Very soluble in methanol, acetone, benzyl alcohol, and methyl acetate. Less soluble in water and ethanol. Insoluble in benzene, ether, butanol, butyl acetate, chloroform, and petroleum ether. Ultraviolet absorption spectrum maxima at 270 to 290 mμ and 420 to 430 mμ (0.1 *N* HCl) or at 270 to 280 mμ and >500 mμ (0.1 *N* NaOH). Negative xanthoproteic, biuret, ninhydrin, Molisch, Fehling, and Sakaguchi tests. Red-brown color with $FeCl_3$. Decolorized by H_2O_2 in presence of Na_2CO_3. Orange-yellow at acid pH, brownish yellow at neutrality, purple-red at pH 7.8, and purple at pH >7.8. Rf = 0.70 by paper chromatography (3 per cent aqueous NH_4OH). C = 54.29%; H = 6.09%; N = 2.95%; Cl = 7.37%; O = 29.3%. $C_{23}H_{29}NO_9 \cdot HCl$. *Reineckate:* Orange needles; m.p. 200–205°C (decomposition) (1, 2, 4, 6, 10).

Biological activity: Active on gram-positive bacteria and mycobacteria. Less active on gram-negative bacteria, except for *Hemophilus*. Very slightly active on fungi (2, 16). Activity reversed by serum (1). Active in mice on *Pneumococcus*, *Brucella melitensis*, *Hemophilus pertussis*, and *Leptospira autumnalis* infections (4, 8). Transient

activity on Yoshida sarcoma and ascites hepatoma (12, 15).

Toxicity: Mice tolerate 6 mg per kg subcutaneously or intravenously, and 108 mg per kg orally (11).

References:

1. Hata, T. *et al.* Kitasato Arch. Exptl. Med. **22:** 229–242, 1949.
2. Hata, T. *et al.* J. Antibiotics (Japan) **3:** 313–325, 1950.
3. Kuroya, M. *et al.* J. Antibiotics (Japan) **4:** 363–366, 1951.
4. Hosoya, S. *et al.* Japan. J. Exptl. Med. **21:** 411–417, 1951.
5. Hosoya, S. *et al.* Japan. J. Exptl. Med. **22:** 313–316, 1952.
6. Hata, T. *et al.* J. Antibiotics (Japan) **5:** 529–534, 1952.
7. Sano, Y. J. Antibiotics (Japan) **5:** 535–538, 1952.
8. Nakase, Y. and Hata, T. J. Antibiotics (Japan) **5:** 542–547, 1952.
9. Hata, T. *et al.* Kitasato Arch. Exptl. Med. **24:** 447–457, 1952.
10. Sano, H. Kitasato Arch. Exptl. Med. **24:** 459–467, 1952.
11. Nakase, Y. and Hata, T. Kitasato Arch. Exptl. Med. **24:** 469–480, 1952.
12. Hosoya, S. *et al.* J. Antibiotics (Japan) **6A:** 42, 1953.
13. Osato, T. *et al.* J. Antibiotics (Japan) **6A:** 52–56, 1953.
14. Kamada, T. J. Antibiotics (Japan) **6A:** 172–181, 1953.
15. Koga, F. J. Antibiotics (Japan) **7A:** 176, 1954.
16. Govorčin, B. Tehnički Pregled Zagreb **8:** 43–52, 1956.

Mannosidostreptomycin

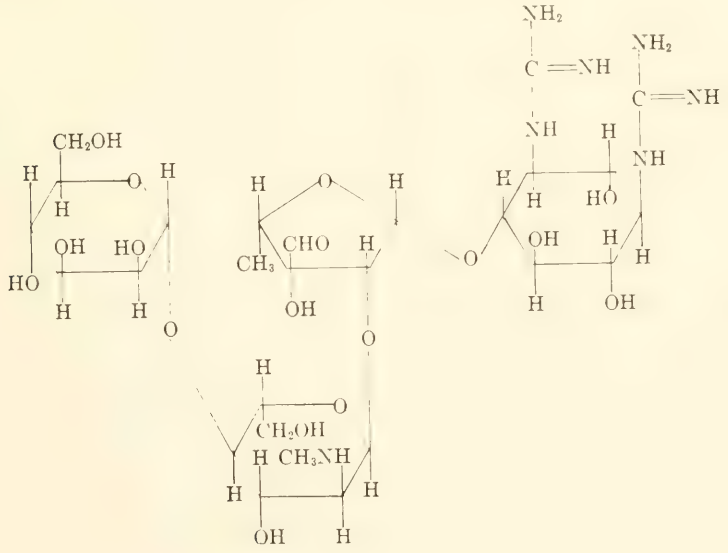
Produced by: Streptomyces griseus (1). This organism also produces cycloheximide, streptocin, and streptomycin (6).

Synonym: Streptomycin B (1).

Remarks: Mannosidostreptomycin is cleaved to streptomycin by the enzyme mannosidostreptomycinase (5).

Method of extraction: Extracted from broth along with streptomycin. Separated by chromatography on acid-washed alumina with 50 per cent methanol as solvent and developer. The more firmly absorbed, less active fractions contain mannosidostreptomycin. Crystallized as the reineckate and converted to the hydrochloride (1, 2). See also II and VI under streptomycin.

Chemical and physical properties: Trihydrochloride: White amorphous powder; m.p. 179–182°C (decomposition) (1) or isotropic hexagonal plates or prisms (dihydrate). $[\alpha]_D^{26.6} = -54.1°$ (c = 1 per cent in water). $pK'_a = 7.6$. *Anhydrous form:* C = 38.25%; H = 6.22%; N = 11.31%; Cl = 12.52%. $C_{27}H_{49}O_{17}N_7 \cdot 3HCl$ (1, 2, 4). *Trireineckate*: Large thin plates containing 8.10 per cent water of crystallization. *Anhydrous form*: m.p. 178–179°C (decomposition, corrected). Structural formula (7):



Dihydrostreptomycin B-HCl: m.p. 194–195°C (decomposition, corrected). $[\alpha]_D^{25} = -55°$ (c = 0.9 per cent in water) (2).

Biological activity: Antimicrobial activity *in vitro* and *in vivo* is qualitatively similar to streptomycin, but quantitatively less active on a weight basis (1, 3, 6).

Toxicity: Intravenous toxicity similar to streptomycin (4).

References:

1. Fried, J. and Titus, E. J. Biol. Chem. **163:** 391–392, 1947.
2. Fried, J. and Stavely, H. E. J. Am. Chem. Soc. **69:** 1549–1550, 1947.
3. Rake, G. *et al.* Proc. Soc. Exptl. Biol. Med. **65:** 107–112, 1947.
4. Heuser, L. J. *et al.* J. Am. Chem. Soc. **70:** 2833–2834, 1948.
5. Perlman, D. and Langlykke, A. F. J. Am. Chem. Soc. **70:** 3968–3969, 1948.
6. Waksman, S. A., ed. Streptomycin; nature and practical applications. The Williams & Wilkins Co., Baltimore, 1949.
7. Fried, J. and Stavely, H. E. J. Am. Chem. Soc. **74:** 5461–5468, 1952.

Matamycin

Produced by: Streptomyces matensis (1).

Synonym: Very closely related to, and probably the same as, althiomycin, differing only in the reaction to Fehling test.

Method of extraction: Broth-filtrate extracted at pH 6.5 to 7.0 with ethyl acetate. Extract concentrated *in vacuo* and cooled to give precipitate. Precipitate taken up in boiling acetone, decolorized, concentrated *in vacuo* and cooled. Recrystallized from ethyl acetate to give a mixture of matamycin and "Compound I," a closely related but antibiotically inactive substance. Purification and separation of the two compounds by countercurrent distribution (acetone-water-ethyl acetate, 2.5:2:2).

Chemical and physical properties: Not strongly acidic or basic. White crystalline substance; m.p. 173°C (decomposition). Very slightly soluble in hot water, dioxane, methanol, ethanol, acetone, and ethyl acetate. Insoluble in petroleum ether. Ultraviolet absorption spectrum shows strong absorption before 220 mμ and a maximum at 285 mμ ($E_{1\,cm}^{1\%}$ 190) (methanol) or at 237 mμ ($E_{1\,cm}^{1\%}$ 550) and 305 to 307 mμ ($E_{1\,cm}^{1\%}$ 270) (in 0.1 *N* NaOH). Infrared spectrum given in reference 2. Unstable at acid pH, being inactivated to the "Compound I" also present in the fermentation broths. $[\alpha]_D = +36.6°$; $[\alpha]_{435} = +57.4°$; $[\alpha]_{365} = +65.4°$ (c = 0.11 per cent in methanol). Positive Tollen, Fehling, bromine, $KMnO_4$, 2,4-dinitrophenylhydrazine, and ninhydrin (after acid hydrolysis) tests. Negative $FeCl_3$, Sakaguchi, and Ehrlich diazo tests. Does not diazotize with nitrous acid. Rf values on chromatography are given in reference 2. Acid hydrolysis products include cysteine, glycine, serine, alanine, questionably arginine, and two unidentified ninhydrin-positive substances. C = 43.95%; H = 4.06%; N = 14.45%; S = 13.57%. No halogen (2).

Biological activity: Active on gram-positive bacteria at 0.5 to 5 μg per ml, *E. coli* at 50 μg per ml, and *K. pneumoniae* at 5 μg per ml. Inactive on *Ps. aeruginosa*, *Pr. vulgaris*, and fungi (1).

References:

1. Margalith, P. *et al.* Antibiotics & Chemotherapy **9:** 71–75, 1959.
2. Sensi, P. *et al.* Antibiotics & Chemotherapy **9:** 76–80, 1959.

Mediocidin

Produced by: Streptomyces mediocidicus (1).

Method of extraction: Mycelium extracted with acetone. Extract evaporated to dryness *in vacuo*. Residue extracted with methanol; ether added to extract to give a precipitate (1).

Chemical and physical properties: Hexaene. Yellow powder. Very soluble in methanol; soluble in ethanol; slightly soluble in water and acetone; insoluble in benzene, ether, and petroleum ether (1). λ_{max}^{MeOH} 339 to 340, 356 to 357, and 377 to 378 (1).

Biological activity: Active mainly on yeasts; moderate activity against certain filamentous fungi and gram-positive bacteria. Slight inhibition of ascites development in mice inoculated with Ehrlich carcinoma (1, 2).

Toxicity: LD_{50} (mice) = 2 mg per kg intraperitoneally (1). Minimal concentration causing HeLa cell degeneration is 200 μg per ml (3).

References:

1. Utahara, R. *et al.* J. Antibiotics (Japan) **7A:** 98–103, 120–124, 1954.
2. Nitta, K. *et al.* J. Antibiotics (Japan) **8A:** 120–125, 1955.
3. Nitta, K. Japan. J. Med. Sci. & Biol. **10:** 277–286, 1957.

Megacidin

Produced by: Streptomyces sp. resembling, but not identical to, *S. fradiae*.

Method of extraction: Broth extracted with ethylene chloride. Extract concentrated *in vacuo*. Residue taken up in benzene. Benzene shaken with acetic acid and concentrated to dryness.

Purification by chromatography on alumina from benzene. Elution with benzene, chloroform, and chloroform-methanol (50:1) (active fraction). Rechromatographed on alumina and eluted with chloroform. Subjected to countercurrent distribution (methanol-water-carbon tetrachloride-chloroform, 4:1:3:2). Water added to active fractions; extracted with chloroform. Extract taken to dryness. Residue extracted with ethyl acetate and precipitated with ether. Recrystallization from ethyl acetate-ether.

Chemical and physical properties: Neutral substance. Colorless thin plates. $C_{24}H_{38}O_{10}$: C = 59.27%; H = 7.94%; active H = 0.42%; O—CH_3 = 6.52%; C—CH_3 = 9.38%. Ultraviolet absorption spectrum maximum at 217 mμ (log ε = 3.94). Infrared absorption spectrum given in reference 1. Positive $FeCl_3$ test. Hydrogenation product has no ultraviolet absorption spectrum maximum at 217 mμ. May contain three to four C—CH_3 groups, and an α,β-unsaturated carbonyl group. Forms a monoacetyl derivative: colorless crystals; m.p. 218.5–220°C.

Biological activity: Very narrow range of activity: *B. megaterium* (0.1 to 1 μg per ml) and *Streptococcus pyogenes* (10 μg per ml). Other gram-positive bacteria, including streptococci, *Staph. aureus*, and mycobacteria, gram-negative bacteria, and yeasts are not affected. No activity on *Streptococcus pyogenes* infection in mice.

Toxicity: Mice tolerate 0.5 gm per kg subcutaneously.

Reference: 1. Ettlinger, L. *et al.* Monatsh. Chem. **88:** 989–995, 1957.

Melanomycin

Produced by: Streptomyces melanogenes (2).

Method of extraction: Broth-filtrate adjusted to pH 4.0 and passed through a column of IRC-50 resin (H^+ form) and the active fraction eluted with 1 to 2 *N* NH_4OH. Addition of 10 per cent acetic acid to pH 3.0 produces a blackish precipitate. An aqueous solution of this material (pH 8.0 with 4 per cent NaOH) is dialyzed against tap water overnight, then lyophilized. Another method of extraction involves the use of IR4B (Cl^- form) resin and elution with aqueous alkali. The antibiotic can be precipitated with zinc chloride, potassium alum, ammonium sulfate, trichloroacetic, picric, and phosphotungstic acids, ammonium reineckate, and other agents. Not precipitated by methyl orange.

Chemical and physical properties: Tasteless, black-brownish, amorphous powder. Amphoteric. Insoluble in acidic water and organic solvents. Soluble in water at pH 6 to 9 and in methanol at acid or alkaline pH. Soluble with inactivation in acetone, butanol, and ethanol containing more than 10 per cent alkaline water. Nondialyzable. Positive xanthoproteic, diazo, sodium nitroprusside, and Millon tests. Acid hydrolysis products include phenylalanine, leucine, proline, alanine, arginine, histidine, glutamic acid, and glycine. Ultraviolet absorption spectrum shows an indistinct shoulder at 260 to 270 mμ. Isoelectric point about pH 3.0. Na melanomycin: C = 54.74%; H = 6.99%; N = 9.87%; Na = 1.9%; Kjeldahl N = 9.00%. Activity decreased by 50 per cent after heating to 100°C for 10 minutes. Hemolyzes horse red blood cells at >124 μg per ml (1).

Biological activity: Mild but definite effect against Ehrlich carcinoma, both ascitic and subcutaneous forms. No antibacterial or antifungal activity (1). Anti-ascarid activity (ova) (3).

Toxicity: Maximal tolerated dose = 250 mg per kg subcutaneously, 50 mg per kg intravenously, and 125 mg per kg intraperitoneally (1).

References:

1. Sugawara, R. *et al.* J. Antibiotics (Japan) **10A:** 133–137, 1957.
2. Sugawara, R. and Onuma, M. J. Antibiotics (Japan) **10A:** 138–142, 1957.
3. Takaoka, M. *et al.* J. Antibiotics (Japan) **11A:** 134–137, 1958.

Melanosporin

Produced by: Streptomyces melanosporus var. *melanosporofaciens.*

Remarks: The same organism produces elaiophylin.

Method of extraction: Extraction of the mycelium with n-butanol. Concentration of butanol to syrup. Addition of ether to syrup yields a yellowish precipitate. White crystals of elaiophylin obtained from the mother liquor, on standing. The precipitate contains melanosporin, elaiophylin, and other antibiotics, including a polyene. *Elaiophylin* extracted with chloroform, followed by evaporation and crystallization from aqueous ethanol. *Melanosporin* is left in the residue, which is suspended in anhydrous ethanol, stirred, and filtered. Addition of ether to the ethanol precipitates impure melanosporin. Further purification by countercurrent distribution in chloroform - methanol - water (2:2:1).

Chemical and physical properties: Very light yellow amorphous solid; m.p. 132–134°C. Soluble in alcohols and dimethylformamide. Insoluble in acetone, chloroform, and ether. $[\alpha]_D^{20} = +30°$ (c = 1.578 per cent in methanol). Light-absorption

maximum at 230 mμ in methanol. Tentative empirical formula: $C_{60}H_{111}O_{21}N_3$. Yellow color, turning brown with sulfuric acid. Brown, nonspecific Molisch test. Negative Fehling, $FeCl_3$, and ninhydrin tests. Strong acid hydrolysis yields three ninhydrin-positive fragments. Infrared absorption spectrum given in reference 1.

Biological activity: Active against gram-positive bacteria and fungi.

Toxicity: LD_{50} (mice) 15 mg per kg intraperitoneally, 350 mg per kg orally.

Reference: 1. Arcamone, F. M. *et al.* Giorn. microbiol. **7:** 207–216, 1959.

Mesenterin

Produced by: Nocardia mesenterica.

Method of extraction: Extraction of broth at pH 7.4 with butyl acetate. Concentration to small volume under vacuum. Concentrate cooled; crude crystals of azomycin precipitate and are removed. The concentrate then extracted with water at pH 2.0. Aqueous layer adjusted to pH 8.0 and extracted with benzene. Evaporation of the benzene leaves a crude white amorphous powder, which is dissolved in methanol and treated with active carbon. Filtrate evaporated to dryness and the residue dissolved in benzene. Benzene solution chromatographed over a column of alumina. Elution with benzene yields antibiotic 446; further elution with benzene and acetone yields mesenterin.

Chemical and physical properties: Basic, colorless substance. Crystallized as needles; m.p. 122–126°C. Strong end-absorption of ultraviolet light in ethanolic solutions. C = 65.82%; H = 7.10%; N = 8.66 to 8.44%. No halogen or S. Negative ninhydrin, Millon, biuret, Tollen, Fehling, and $FeCl_3$ tests. Positive Molisch reaction. Very soluble in methanol, ethanol, ethyl acetate, butyl acetate, and ether. Soluble in benzene and acidic water. Slightly soluble in water. Insoluble in petroleum ether. A 10 per cent loss of activity at pH 6.0 upon heating for 30 minutes at 100°C in aqueous solution.

Biological activity: Active *in vitro* against gram-positive bacteria, including mycobacteria. No activity against gram-negative bacteria.

Toxicity: LD_{50} >100 mg per kg. (Route of injection and animal used not reported.)

Reference: 1. Ueda, M. and Umezawa, H. J. Antibiotics (Japan) **8A:** 164–167, 1955.

Methymycin

Produced by: Streptomyces sp. (1) and *S. eurocidicus* (10).

Synonym: Antibiotic 11 B (10).

Remarks: Belongs to the erythromycin-carbomycin-proactinomycin group. Differs from picromycin only in point of attachment of desosamine to the lactone ring.

Method of extraction: Broth-filtrate extracted with amyl acetate at pH 9.5. Back-extracted into dilute sulfuric acid. Acid washed with chloroform at pH 2.5, then extracted with it at pH 9.5. Concentration of extract and dilution of concentrate with Skellysolve C gives needles of methymycin. Recrystallized from absolute ethanol (1). Purified by chromatography. Crystallized from ethyl acetate (5).

Chemical and physical properties: Macrolide (9). Needles, m.p. 203–205°C (from ethyl acetate); or a polymorphic modification (prisms from ethanol), m.p. 195.5–197°C (5). *Free base*: Soluble in methanol, acetone, chloroform, and dilute acids; moderately soluble in ethanol and ether; insoluble in water and hexane. Ultraviolet absorption spectrum maxima at 223 to 225 mμ (ϵ = 10,500) and at 322 mμ (ϵ = 47) (ethanol) (1, 5). Infrared spectrum given in reference 1. $[\alpha]_D^{25}$ = +61° (c = 0.7 per cent in methanol). Positive permanganate and Br tests. Negative Molisch, biuret, ninhydrin, and $FeCl_3$ tests. pK_b = 5.7 (1, 6). Rf values in various systems (paper chromatography) given in reference 3. *Sulfate:* needles; m.p. 173–178°C. Soluble in methanol and water (1). *2,4-Dinitrophenylhydrazone:* Orange needles; m.p. 205–207°C (5). *Quaternary salt with methyl iodide:* $C_{26}H_{46}NO_7I$; m.p. 190°C. $[\alpha]_D$ = +31.0° (ethanol) (7). Mild acid hydrolysis products include desosamine (6), also found in erythromycin. Oxidation with $KMnO_4$ in acetone yields the lactone of β-hydroxy-α,α′,γ-trimethylpimelic acid. Both products are also produced from narbomycin and picromycin (8). Formula of methymycin: $C_{25}H_{43}NO_7$. C = 63.93%; H = 9.28%; N = 2.84%; 2N—CH_3 = 5.83%; 6C—CH_3 = 18.3% (5). Complete structure (6) given in Chapter 6.

Biological activity: Active against certain strains of gram-positive bacteria and a few strains of gram-negative bacteria (*Malleomyces mallei*, *Br. suis*, and *Pasteurella tularensis*). No antifungal activity (1). Not active on *Toxoplasma gondii* infections in mice (4).

Utilization: Suppressive but not curative effect on clinical brucellosis (2).

References:

1. Donin, M. N. *et al.* Antibiotics Ann. 179–185, 1953–1954.
2. Max, G. C. and Mendez, D. Antibiotics & Chemotherapy **4:** 83–86, 1954.

3. Sokolski, W. T. *et al.* Antibiotics & Chemotherapy **4:** 1057–1060, 1954.
4. Palencia, L. *et al.* Rev. inst. salubridad y enfermedad trop. (Mex.) **14:** 113–116, 1954.
5. Djerassi, C. *et al.* J. Am. Chem. Soc. **78:** 1729–1732, 1956.
6. Djerassi, C. and Zderic, J. A. J. Am. Chem. Soc. **78:** 6390–6395, 1956.
7. DeSomer, P. Giorn. microbiol. **2:** 216–232, 1956.
8. Anliker, R. *et al.* Helv. Chim. Acta **39:** 1785–1790, 1956.
9. Woodward, R. B. Angew. Chem. **69:** 50–58, 1957.
10. Taguchi, H. and Nakano, A. J. Fermentation Technol. **35:** 191–195, 1957.

Miamycin

Produced by: Streptomyces sp. resembling *S. ambofaciens.*

Remarks: Belongs to the erythromycin-like group. Culture also produces spiramycin.

Method of extraction: Broth extracted with chloroform, n-butanol, or ethyl acetate at pH 8.5. Purification by repeated extraction into water at pH 4.5 and back-extraction into solvent at pH 8.5, then fractional precipitation from a mixture of ether-petroleum ether. Crystallized from ether. Further purification by countercurrent distribution (ethyl acetate-0.1 *M* phosphate buffer pH 6.9).

Chemical and physical properties: Soluble in lower alcohols, dilute acids, chloroform, acetone, ethyl acetate, and benzene. Slightly soluble in water, carbon disulfide, carbon tetrachloride, and petroleum ether (40–60°C); m.p. 221–222°C (decomposition). $[\alpha]_D^{25} = -18°$ (c = 1 per cent in 0.02 *N* hydrochloric acid). Optically inactive in 0.02 *N* HCl. Ultraviolet absorption spectrum maximum at 230 mμ (broad peak). Infrared absorption spectrum given in reference 1. Decolorizes 2 per cent $KMnO_4$ in acetone in the cold but does not react with Br in CCl_4. Positive Molisch and Elson-Morgan tests. Negative ninhydrin test. Dissolves in concentrated H_2SO_4 with a light yellow color which fades on dilution with water. C = 61.45%; H = 8.65%; N (Dumas) = 2.32%. Acid hydrolysis with *N* HCl at 60°C for 24 hours yields two spots when chromatographed on paper strips (water-saturated 10 per cent acetic acid in n-butanol) and sprayed with ammoniacal $AgNO_3$; the spots are identical to those from erythromycin under the same conditions (1). Probably a member of the macrolide group (2).

Biological activity: Active on gram-positive bacteria. Shows cross-resistance with erythromycin and carbomycin. Protects mice against infections with *D. pneumoniae.*

Toxicity: Mice tolerate 750 mg per kg intraperitoneally, and 580 mg per kg orally.

References:

1. Schmitz, H. *et al.* Antibiotics & Chemotherapy **7:** 37–39, 1957.
2. Brink, N. G. and Harman, R. E. Quart. Revs. (London) **12:** 93–115, 1958.

Microcins

Produced by: Micromonospora sp.

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 2.0. Extract shaken with pH 7.0 phosphate buffer. Ethyl acetate layer contains microcin A. Buffer layer re-extracted into ethyl acetate at pH 2.0 to give microcin B.

Chemical and physical properties: Microcin A: Neutral, reddish violet substance. Insoluble in water. *Microcin B:* Acidic, yellowish red substance. Slightly soluble in water. Both substances give negative Molisch and $FeCl_3$ tests.

Biological activity: Active on *Sacch. formosensis* and gram-positive bacteria. Less active on gram-negative bacteria.

Toxicity: LD_{50} (mice) 625 mg per kg intravenously.

Reference: 1. Taira, T. and Fujii, S. J. Antibiotics (Japan) **5:** 185–187, 1952.

Micromonosporin

Produced by: Micromonospora sp. (1).

Method of extraction: Precipitated, with loss of activity, by 50 to 90 per cent ethanol or acetone, and without loss of activity by saturation of broth-filtrate with $(NH_4)_2SO_4$ to 50 to 75 per cent (3). Chocolate-brown precipitate dissolved in 5 per cent NaCl solution and dialyzed against tap water, then distilled water. Solution lyophilized. Could also be adsorbed on Norit, but not eluted with aqueous buffer solutions or common organic solvents (2).

Chemical and physical properties: Highly pigmented protein, probably associated with a carbohydrate moiety. Water-soluble. Insoluble in ether, alcohol, or acetone. Destroyed by concentrated acid, at high pH, or by heat. Not destroyed by pepsin or trypsin. Positive Molisch test. Negative phloroglucinol, orcinol, and naphthoresorcinol reactions. N = 6.7%. After acid hydrolysis, gives 5.5 per cent amino N (1–3).

Biological activity: Active on gram-positive bac-

teria. Not active on *B. cereus-mycoides* group. No activity on gram-negative bacteria (1, 3).

References:

1. Waksman, S. A. *et al.* Soil Sci. **54:** 281–296, 1942.
2. Waksman, S. A. *et al.* J. Bacteriol. **53:** 355–357, 1947.
3. Welsch, M. Rev. belge pathol. et méd. exptl. Suppl. II: 18, 1947.

Mikamycin A

Produced by: Streptomyces mitakaensis (1).

Synonyms: Mikamycin. The main components of streptogramin and staphylomycin are thought to be the same as those of mikamycin (1).

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 6.0. Extract washed with water, with dilute HCl at pH 2.0, and with $NaHCO_3$ at pH 7.4. Solvent layer concentrated *in vacuo* at 27–30°C. Addition to a large volume of petroleum ether precipitates mikamycin. Powder dissolved in methanol, filtered, and solution concentrated. Concentrate washed with ether, and extracted with chloroform. Chloroform solution chromatographed on HNO_3-treated alumina in carbon tetrachloride. Washed with benzene and ethyl acetate. Eluted with methanol. Active fractions concentrated and precipitated from ether. Purification by countercurrent distribution (methanol - benzene - chloroform - water, 20:25:15:7). The solvent of the active fractions evaporated *in vacuo* and the aqueous residue extracted with chloroform. Extract concentrated to dryness. Redistributed countercurrently (methanol-benzene-chloroform-water, 27:28:12:8). Active fractions crystallized from benzene (1).

Chemical and physical properties: Neutral. Yellowish white crystalline substance; m.p. 178°C (decomposition). $C_{31}H_{39}O_9N_3$: C = 62.08%; H = 6.58%; N (Dumas) = 7.34%. No halogens or S. Molecular weight, 617. $[\alpha]_D^{28} = -152°$ (c = 0.5 per cent in methanol). Very soluble in chloroform, methanol, ethanol, and acetone. Soluble in methylene chloride, ethylene chloride, benzene, and ethyl acetate. Slightly soluble in ether and water. Insoluble in petroleum ether, carbon tetrachloride, and hexane. Positive Fehling and diazo tests. Negative maltol, glucosamine, ninhydrin, Millon, and biuret tests. Black-green color with $FeCl_3$. Black-brown precipitate with Tollen test (no mirror). Green color and brown precipitate with Benedict test. Labile to alkaline pH; most stable at pH 4.0. Ultraviolet absorption spectrum (methanol) maximum at 226 mμ ($E_{1\,cm}^{1\%}$ 624) and an inflection at 270 mμ ($E_{1\,cm}^{1\%}$ 200). In 0.1 *N* NaOH-4 per cent methanol, a maximum at 293 mμ ($E_{1\,cm}^{1\%}$ 462) shifts to 283 mμ ($E_{1\,cm}^{1\%}$ 201) after inactivation at this pH. Acidification causes this peak at 283 mμ to disappear. It reappears on readjustment to alkaline pH. Staphylomycin and streptogramin react similarly. Forms a dinitrophenylhydrazone: m.p. 160–165°C (decomposition). Infrared data given in references 2 and 3. Paper chromatography (3 per cent NH_4Cl) shows one spot (Rf = 0.59) for mikamycin, two spots (Rf = 0.60 and 0.39) for streptogramin, and two spots (Rf = 0.61 and 0.41) for staphylomycin (assay organism, *Sarcina lutea*). Hydrogenation yields *decahydromikamycin*, which has no absorption of ultraviolet light: white plates; m.p. 94°C (decomposition). Contains 2C—CH_3 , 1N—CH_3 , 2C = O, and 5C = C. *Monoacetate:* Biologically inactive, yellow-green crystals; m.p. 138°C. Ultraviolet absorption spectrum maximum at 236 mμ (ε = 42,347) and an inflection at 270 mμ (ε = 21,621). *2,4-Dinitrophenylhydrazone:* Red crystals; m.p. 180–186°C (decomposition). Acid hydrolysates of mikamycin contain L-proline and glycine. An additional weakly ninhydrin-positive spot was assigned to an N-methyl-containing moiety. Mikamycin may contain a chromophore attached to a peptide chain (1).

Biological activity: Active on gram-positive bacteria (0.1 to 55 μg per ml); less active on mycobacteria (20 to 28 μg per ml) (1); not active on gram-negative bacteria, fungi, or yeasts. Active in mice on *D. pneumoniae* infections (1).

Toxicity: Mice tolerate 250 mg per kg intraperitoneally (1).

References:

1. Arai, M. *et al.* J. Antibiotics (Japan) **11A:** 14–20, 1958.
2. Arai, M. *et al.* J. Antibiotics (Japan) **11A:** 21–25, 1958.
3. Okabe, K. J. Antibiotics (Japan) **12A:** 86–89, 1959.
4. Watanabe, K. *et al.* J. Antibiotics (Japan) **12A:** 112–113, 1959.

Mikamycin B

Produced by: Streptomyces mitakaensis.

Synonym: Similar to antibiotic PA 114B.

Remarks: Mikamycin was described as probably identical with streptogramin and antibiotic 899. This compound should now be called mikamycin A, since another antibiotic has been found to be produced by the same actinomycete and has been called mikamycin B. As previously observed for similar antibiotics, such as PA 114, staphylo-

mycin, and antibiotic 129, a synergistic relationship was found between mikamycins A and B (2).

Method of extraction: Extraction of broth-filtrate with ethyl acetate. Concentration of the ethyl acetate extract under vacuum to $\frac{1}{20}$ volume. A precipitate forms which contains 48 per cent mikamycin A and 6.5 per cent mikamycin B. Addition of 10 volumes of petroleum ether to the supernatant gives a syrup containing 13 per cent mikamycin A and 18 per cent mikamycin B. Syrup dissolved in methanol; addition of ether causes formation of a precipitate, which is dried *in vacuo*. Resulting powder dissolved in benzene and chromatographed over silicic acid. Column washed with chloroform, and elution carried out with chloroform containing 5 per cent acetone. Fractions of the eluate containing only mikamycin B (Rf = 0.05, ascending paper chromatography with 3 per cent ammonium chloride) combined and concentrated to a syrup *in vacuo*. Syrup treated with ether. A white insoluble powder contains 72 per cent mikamycin B and less than 1 per cent mikamycin A. This powder extracted with ether in a Soxhlet apparatus. Crude crystals of mikamycin B precipitated from the ether solution. Recrystallized from methanol.

Chemical and physical properties: Amphoteric, white, platelet-shaped crystals, which melt at 160°C, solidify above 180°C, and decompose at 262°C. $[\alpha]_D^{15} = -61.3°$ (c = 1.0 per cent in methanol). Molecular weight (Signer), 865. C = 60%; H = 6.43%; N = 12.53%. Suggested empirical formula: $C_{45}H_{58}N_8O_{11}$. Light-absorption maxima at 209, 260, and 305 mμ in methanol. Infrared absorption spectrum given in reference 1. Soluble in chloroform, acetone, methyl isobutyl ketone, benzene, ethyl acetate, ethanol, and methanol. Slightly soluble to insoluble in ether, cyclohexane, petroleum ether, and water. Salts are soluble in water. Positive $FeCl_3$ (brown-red) test. Mikamycin A gives a dark green $FeCl_3$ test. Almost negative ninhydrin reaction. Negative Ehrlich, biuret, Fehling, and Tollen reactions. Stable at neutral and acidic reactions (1).

Mikamycin is a macrocyclic peptide lactone composed of the following seven amino acids: 3-hydroxypicolinic acid, L-threonine, D-α-amino-*n*-butyric acid, L-proline, L-phenylglycine, L-4-oxopipecolic acid, and L-*p*-dimethyl amino-N-methylphenylalanine. This last amino acid had not been previously reported (4). The structure given in reference (5) is closely related to those of etamycin and staphylomycin S.

Biological activity: Active against gram-positive bacteria. Mikamycins A and B have synergistic activity *in vitro* and *in vivo* when there is 10 to 90 per cent of mikamycin B in the mixture (2). Mikamycin B inhibits *Staph. aureus* at 8 μg per ml (A at 2 μg per ml); mikamycin A inhibits *Sarcina lutea* at 0.25 μg per ml (B at 4 μg per ml); *B. subtilis* is inhibited by mikamycin A at >64 μg per ml (B at 8 μg per ml). *Staph. aureus* exposed to mikamycin B becomes more sensitive to A, but the reverse is not true (3).

Toxicity: Mice tolerate 350 μg per ml intraperitoneally (3).

References:

1. Watanabe, K. J. Antibiotics (Japan) **13A:** 57–61, 1960.
2. Watanabe, K. J. Antibiotics (Japan) **13A:** 62–69, 1960.
3. Watanabe, K. *et al.* J. Antibiotics (Japan) **12A:** 112–113, 1959.
4. Watanabe, K. J. Antibiotics (Japan) **14A:** 1–13, 1961.
5. Watanabe, K. J. Antibiotics (Japan) **14A:** 14–17, 1961.

Miramycin

Produced by: Streptomyces mirabilis.

Method of extraction: Unknown. Culture-broths contain more than one antibiotic.

Chemical and physical properties: Heat-stable.

Biological activity: Active against gram-positive and gram-negative bacteria.

Toxicity: Said to be nontoxic.

References:

1. Ruschmann, G. Pharmazie **7:** 542–550, 639–648, 823–831, 1952.
2. Böing J. Pharmazie **13:** 305–310, 1958.

Mitomycins

Produced by: Streptomyces caespitosus (2, 9) and *S. griseovinaceseus* (3).

Remarks: In the hands of one group of investigators (9), *S. caespitosus* produced a complex differing somewhat from that originally described (1). Further fractions other than those described below have been reported, but no details are known (12).

Methods of extraction: Original description: Filtered broth treated with activated carbon at pH 6.0 to 6.5. Eluted with acetone. Acetone concentrated *in vacuo* and the residue extracted with chloroform (1). Purple-red chloroform extract dehydrated with anhydrous Na_2SO_4 and chromatographed on alumina. Colored fractions included: yellow, blue-violet, pink and blue, red-violet, and purple. The latter two, mitomycins A and B, eluted with methanol or water. Puri-

fied by rechromatographing on alumina from chloroform, with chloroform or chloroform-acetone as developer. Recrystallized from acetone on addition of carbon tetrachloride in the cold. Mitomycins A and B could also be separated by countercurrent distribution (benzene-chloroform and pH 7.2 phosphate buffer) (1). Mitomycin C was also distinguished (4). The later workers used a method of extraction essentially the same as that given above except that "Fraction R" was described as being left in the aqueous layer after step I. This was extracted from the aqueous layer with cyclohexanone, the extract concentrated, and the substance precipitated with petroleum ether. Chromatography gave active fractions described as mitomycins A, B, and C(X), and fractions X and Y (9).

Chemical and physical properties: Mitomycins A and B: Basic substances. Plates or needles. Soluble in acetone, methanol, ethanol, chloroform, methyl ethyl ketone, pyridine, methyl Cellosolve, n-butanol, ethylene dichloride, water, ethyl and n-butyl acetate, ether, and cyclohexanol. Slightly soluble or insoluble in xylene, carbon disulfide, carbon tetrachloride, ligroin, petroleum ether, and cyclohexane. Infrared spectra given in reference 1. Positive 2,4-dinitrophenylhydrazine and bromine tests. Negative Elson-Morgan, biuret, and anthrone tests. *Mitomycin A:* m.p. 159–161°C (decomposition). Soluble in benzene, toluene, trichloroethylene, and nitrobenzene. Light-absorption maxima at 215 mμ ($E^{1\%}_{1\,cm}$ 234), 316 to 318 mμ ($E^{1\%}_{1\,cm}$ 122), and 530 mμ ($E^{1\%}_{1\,cm}$ 18.8) (in water); at 360 and 550 to 560 mμ (in 0.1 *N* NaOH); or at 235, 285, 335, and 430 mμ (in 0.1 *N* HCl). Red-violet color of mitomycin A fades in Schiff's reagent, 0.1 *N* HCl, and 0.1 *N* NaOH; changes to blue in concentrated H_2SO_4 and to orange in concentrated HCl. Gives a green color with Molisch reagent. C = 54.22%; H = 5.05%; N = 11.68%. No Cl or S. *Mitomycin B:* m.p. 182–184°C (decomposition). Insoluble in benzene, toluene, trichloroethylene, and nitrobenzene. Light-absorption maxima at 220 mμ ($E^{1\%}_{1\,cm}$ 117.5), 320 mμ ($E^{1\%}_{1\,cm}$ 55), and 550 mμ ($E^{1\%}_{1\,cm}$ 9.9) (in water); at 360 and 580 mμ (0.1 *N* NaOH); and at 232, 284, 335, and 430 to 440 mμ (0.1 *N* HCl). The violet color of mitomycin B fades with Schiff's reagent and 0.1 *N* HCl; changes to blue with 0.1 *N* NaOH, and from blue to green with concentrated H_2SO_4. Color changes from yellow to orange with concentrated HCl, and from green to brown with the Molisch reagent (1). *Mitomycin A-like:* Red crystals; m.p. 167–168°C. Light-absorption maxima at 216 mμ ($E^{1\%}_{1\,cm}$ 485), 320 mμ ($E^{1\%}_{1\,cm}$ 255), and 520 mμ ($E^{1\%}_{1\,cm}$ 40) in methanol. Pure substance retains 93 per cent of its activity after 3 hours at 100°C. Less pure preparations are more labile. C = 51.46%; H = 5.64%; N = 9.00%; O = 33.90%. Degradation products include cinnamic acid amide. *Mitomycin C* (*X*): Bluish violet crystals. No melting point up to 360°C. Soluble in water, methanol, acetone, butyl acetate, and cyclohexanone. Sparingly soluble in benzene, carbon tetrachloride, and ether. Insoluble in petroleum ether. Ultraviolet absorption spectrum maxima at 216 mμ ($E^{1\%}_{1\,cm}$ 742), 360 mμ ($E^{1\%}_{1\,cm}$ 742), and 560 mμ ($E^{1\%}_{1\,cm}$ 0.06). Infrared spectrum given in reference 9. Positive Fehling, hydroxylamine hydrochloride, biuret, Ehrlich, $FeCl_3$, nitrous acid, and Liebermann tests. Questionable positives: bromine, 2,4-dinitrophenylhydrazine, and Janovsky reactions. Negative Benedict, Tollen, fuchsin, ninhydrin, Millon, and Raymond tests. Data on paper chromatographic behavior given in reference 9. Bluish violet at alkaline pH, red at weakly acid pH, and yellow at acid pH. Color changes are reversible. Most stable at pH 6 to 7; labile to acid and alkali. Photo-labile. Prolonged boiling destroys biological activity and toxicity. Easily oxidized, but not easily reduced. C = 53.84%; H = 5.14%; N = 15.49%. $C_{54}H_{61}N_{13}O_{19}$ (tentative). *Chromophore of C:* Isolation procedure given in reference 9. Red-brown crystals; m.p. 65–70°C (not distinct). Soluble in water, methanol, ethanol, acetone, cyclohexanone, and dioxane. Ultraviolet absorption spectrum maxima at 215 mμ ($E^{1\%}_{1\,cm}$ 630) and 320 mμ ($E^{1\%}_{1\,cm}$ 295) with a shoulder at 260 mμ. Exhibits the same color changes as mitomycin C. Biologically inactive. *Fraction R:* Brownish red amorphous powder. Soluble in acetone, butanol, methanol, dioxane, and alkaline water. Insoluble or slightly soluble in ethyl acetate, butyl acetate, chloroform, carbon tetrachloride, ether, benzene, and water. Ultraviolet absorption spectrum maxima at 215 and 315 mμ. Does not change color with pH. *Fraction Y:* Orange crystals. Browns at 180°C. Carbonizes at 240°C. Soluble in chloroform, acetone, benzene, ether, and acidic aqueous solution. Insoluble or slightly soluble in benzene, ether, and neutral or alkaline water. Ultraviolet absorption spectrum maxima at 207, 237, 286, and 345 mμ (9). Violet in alkaline solution; yellow in acidic. Considered a partial degradation product of mitomycin B.

Biological activity: Mitomycins A and B: Active on gram-positive and gram-negative bacteria, mycobacteria, and *Nocardia asteroides*. Not active on fungi or yeasts. Mitomycin A is active in a

range from 0.0005 to 1.0 μg per ml; B, from 0.05 to 5.0 μg per ml. Partial cross-resistance with streptomycin (1, 5). Inactive in mice on *D. pneumoniae, Sal. enteritidis*, and *Leptospira icterohaemorrhagiae*. Active on *Rickettsia tsutsugamushi*. Inactive on *Miyagawanella* and viruses (6). Both are active on Yoshida sarcoma in rats and Ehrlich carcinoma (ascitic form) in mice (1, 4). Mitomycins A and B are equally active against tumors; C is said to be about one fourth to one fifth as active. Some effect on the solid form of Ehrlich carcinoma (4). *Mitomycin A-like:* Same as mitomycins A and B (9). *Mitomycin C:* Same *in vitro* activity as A and B (9), but only one twentieth as active as A on *B. subtilis* (4). Active *in vivo* on *D. pneumoniae, Sal. enteritidis, L. icterohaemorrhagiae, R. tsutsugamushi*, and *Miyagawanella* (sheep). Inactive on toxoplasmosis (mice) or infections caused by other small viruses (5, 6). Antiascarid activity (12). Active on the following neoplasms: sarcoma 180 (ascitic), Ehrlich carcinoma (ascitic), adenocarcinoma EO 771, Bashford carcinoma 63, Miyono adenocarcinoma, carcinoma 1025, Wagner osteogenic sarcoma, Ridgeway osteogenic sarcoma, Lewis lung carcinoma, Harding-Passey melanoma, glioma 26, Friend virus leukemia, Flexner-Jobling carcinoma, Walker carcinosarcoma 256, Gardiner lymphosarcoma, Jensen sarcoma, Murphy-Sturm lymphosarcoma, Crabb hamster sarcoma, Iglesias functional rat adrenal tumor, Iglesias functional rat ovarian tumor, diploid and tetraploid Hirosaki sarcoma (ascites and solid), Usubuchi sarcoma (ascitic), sarcoma-3-B (ascitic), ascites hepatoma 7974, Yoshida sarcoma, and mouse lymphatic leukemia SN36 (7, 10, 13). May interfere with the *de novo* synthesis of purines (11). *Fraction R:* Moderately active on Ehrlich carcinoma (ascitic), but not on bacteria (9). *Fraction Y:* Same as mitomycins A and B (9).

Toxicity: Mitomycin A: LD_{50} (mice) 1.0 to 1.5 mg per kg intravenously (5). *Mitomycin A-like:* LD_{50} (mice) 2.5 mg per kg intravenously. *Mitomycin C:* More toxic for rats and hamsters than for mice (13). LD_{50} (mice) 5 mg per kg (9) or 7.5 to 10.0 mg per kg (5) intravenously, 9 mg per kg intraperitoneally (9). Toxicity also studied in rats and rabbits (14). *Fraction R:* LD_{50} >500 mg per kg (no route given) (9).

Utilization: Mitomycin C(X) has been reported clinically effective in certain neoplastic diseases (8, 12).

References:

1. Hata, T. *et al.* J. Antibiotics (Japan) **9A:** 141–146, 1956.
2. Sugawara, R. and Hata, T. J. Antibiotics (Japan) **9A:** 147–151, 1956.
3. Japanese Patent 2,898, April 17, 1956.
4. Kanamori, H. *et al.* J. Antibiotics (Japan) **10A:** 120–127, 1957.
5. Matsumae, A. *et al.* Japan. J. Microbiol. **1:** 183–189, 1957.
6. Saito, Y. *et al.* Japan. J. Microbiol. **1:** 191–196, 1957.
7. Usubuchi, I. *et al.* Gann **48:** 447–448, 1957.
8. Sakai, K. Chemotherapy (Tokyo) **5:** 322, 1957.
9. Wakaki, S. *et al.* Antibiotics & Chemotherapy **8:** 228–240, 1958.
10. Usubuchi, I. *et al.* Chemotherapy (Tokyo) **6:** 378–392, 1958.
11. Reilly, H. C. and Cappuccino, J. G. Proc. Am. Assoc. Cancer Research **2:** 338, 1958.
12. Shiraha, Y. *et al.* Antibiotics Ann. 533–540, 1958–1959.
13. Sugiura, K. Cancer Research **19:** 438–445, 1959.
14. Sokoloff, B., *et al.* Growth **24:** 1–27, 1960.

Moldcidin A

Produced by: Streptomyces sp.

Remarks: Moldcidin B, another pentaene, has been shown to be identical with pentamycin.

Method of extraction: Extraction of the mycelium with methyl alcohol. Concentration of solvent *in vacuo* to 1/10 volume. Precipitate collected and dried. Extract dissolved in methyl alcohol, filtered, water added. Upon concentration, crystals precipitate.

Chemical and physical properties: Pentaene. Light-absorption maxima at 324, 339, and 358 mμ. Insoluble in butanol, ether, petroleum ether, ethyl acetate, and 0.01 *N* HCl. Slightly soluble in water, acetone, ethyl alcohol, and 0.1 *N* NaOH. Soluble in methyl alcohol, 80 per cent acetone, glacial acetic acid, and pyridine. Positive ninhydrin reaction. Negative biuret, Fehling, Molisch, Sakaguchi, and $FeCl_3$ tests. Slow melting between 180 and 230°C. No loss of activity of 1 mg per ml in methyl alcohol at 5°C during 24 hours. Inactivated by ultraviolet light. C = 55.87%; H = 8.84%; N = 1.50%.

Biological activity: Active against a number of yeasts and filamentous fungi at levels of 0.5 to 30 μg per ml. No activity against bacteria. Active against *Trichomonas vaginalis*.

Toxicity: LD_{50} (mice) 10 mg per kg intravenously.

Utilization: Active against *C. albicans* and *T. vaginalis* when applied topically in the vagina.

References:

1. Sakamoto, J. M. J. J. Antibiotics (Japan) **12A:** 169–172, 1959.
2. Ogawa, H. *et al.* J. Antibiotics (Japan) **13A:** 353–355, 1960.

Moldin

Produced by: Streptomyces phaeochromogenes (1, 2).

Method of extraction: Broth extracted with ethyl acetate. Extract concentrated *in vacuo*. Syrup washed with petroleum ether, water, and hot water, and then dried. Residue dissolved in ethanol and precipitated on addition of distilled water. Antibiotic is also present in mycelium (1), which can be extracted with ethanol (2).

Chemical and physical properties: Soluble in ethanol and ethyl acetate. Slightly soluble in petroleum ether, ether, and benzene. Almost insoluble in water. Positive Molisch and $FeCl_3$ tests. Negative biuret, Millon, ninhydrin, Tollen, Fehling, and Sakaguchi tests (1, 2). Ultraviolet absorption spectrum maximum at 320 mμ.

Biological activity: Active on yeasts (1).

Toxicity: MLD is 10 mg per kg intraperitoneally (1).

References:

1. Maeda, K. *et al.* J. Antibiotics (Japan) **5:** 465, 1952.
2. Maeda, K. *et al.* Japan. J. Med. Sci. & Biol. **5:** 327–339, 1952.

Monamycin

Produced by: Streptomyces jamaicensis.

Method of extraction: Extraction of culture-filtrate or mycelium with ether or butanol. Evaporation of solvent. Countercurrent distribution using ethyl acetate-cyclohexane-methanol-water (12:10:10:7) followed by countercurrent distribution using light petroleum (b.p. 60–80°C)-methanol-water (10:10:1). Chromatography on Amberlite C. G. 4J. Crystallization from light petroleum.

Chemical and physical properties: Needles; m.p. 126°C. Base, giving a crystalline monohydrochloride; m.p. 187°C. $[\alpha]_D^{18} = -62° \pm 5°$ (c = 0.9 per cent in ethanol). Tentative empirical formula: $C_{22}H_{36\text{-}38}N_4O_5$. One N-methyl and three C-methyl groups. End-absorption of ultraviolet light. Infrared spectrum shows no evidence of aromatic structure but suggests the presence of an amide linkage. No reaction with sodium metaperiodate or with hydrogen in presence of platinum catalyst. Stable above pH 7.0.

Biological activity: Active against gram-positive bacteria. Not active against gram-negative bacteria. No cross-resistance with penicillin, chlortetracycline, chloramphenicol, or sulfamethazine, using *Staph. aureus*. Not inactivated by human serum.

Toxicity: 850 mg per kg subcutaneously produces no unfavorable effect in mice.

Reference: 1. Hassall, C. H. and Magnus, K. E. Nature, London **184:** 1223–1224, 1959.

Monilin

Produced by: Streptomyces sp. (1), and *S. sakaiensis* (2).

Synonym: Similar to toyocamycin.

Method of extraction: Broth-filtrate extracted with n-butanol or amyl alcohol at pH 11. Extract washed with water and concentrated *in vacuo* until a precipitate forms. Can also be adsorbed on charcoal from broth and eluted with 90 per cent acetone. Removal of the solvent by vacuum distillation gives an aqueous residue, which is treated as above with butanol. Recrystallized from hot water, ethanol, and methanol. Purified by countercurrent distribution or chromatography (1, 2).

Chemical and physical properties: Basic substance. White needles; m.p. 235–238°C (decomposition). Soluble in methanol, ethanol, butanol, and acetone. Sparingly soluble in water. Insoluble in ethyl acetate, toluene, butyl acetate, benzene, petroleum ether, and ether. Ultraviolet absorption spectrum maxima at 230 and 280 mμ in water. Infrared spectrum given in reference 2. Positive ninhydrin and Sakaguchi tests. $C_{15}H_{20}N_6O_3$. Molecular weight, 190 ± 10. C = 46.93%; H = 5.34%; N = 21.80%.

Biological activity: Active on *C. albicans*, *C. tropicalis*, and *C. parakrusei*. Slightly active on *Sacch. sake*. Not active on *C. krusei* or *C. pseudotropicalis*. Not active on bacteria (1).

Toxicity: LD_{50} (mice) 3.94 mg per kg intraperitoneally (1, 2).

References:

1. Fujii, S. *et al.* Ann. Rept. Takeda Research Lab. **14:** 8–10, 1955.
2. Fujii, S. *et al.* Japanese Patent 5,899, August 3, 1957.

Musarin

Produced by: Actinomyces (*Streptomyces*) sp. (1) having red mycelium. A variant of this culture produces monamycin (4).

Method of extraction: I. Broth extracted with n-butanol at pH 7.0. Extract concentrated *in vacuo*. Addition of ether to concentrate precipitates the antibiotic. II. Addition of $(NH_4)_2SO_4$ to broth; precipitate extracted with cold metha-

nol. Extract concentrated. Addition of ether to concentrate for precipitation. III. Mycelial extract (hot methanol) evaporated to dryness under reduced pressure. Broth-filtrate acidified to pH 3.5 to 4.0 with dilute H_3PO_4. Resulting precipitate and residue from mycelial extraction treated with 1 per cent neutral sodium phosphate buffer. Buffer extracted with n-butanol; butanol evaporated to dryness *in vacuo* at 45°C. Residue taken up in methanol; concentration *in vacuo*. Addition of ether to residual solution gives musarin (3).

Chemical and physical properties: Acid. Yellowish or colorless substance. Sodium or potassium salts soluble in water, methanol, ethanol, and butanol; insoluble in ether and acetone. Acid precipitable with HCl, H_3PO_4, or H_2SO_4 from neutral solution. Forms inactive precipitates with $BaCl_2$, $HgCl_2$, or copper acetate. Free acid is unstable as dry powder; sodium salt is stable. Activity unaltered at pH 2 or 11 at room temperature for 30 minutes, but destroyed at 100°C under these conditions. *Sodium salt:* Decomposes at about 170°C without melting. $[\alpha]_D^{20} = +35.1° \pm 1.6°$ (c = 1.21 per cent in methanol). Ultraviolet absorption spectrum maxima at 240 mμ ($E_{1cm}^{1\%}$ 375) and 267 mμ ($E_{1cm}^{1\%}$ 200) (ethanol). *Free acid:* C = 57.75%; H = 8.34%; N = 3.70%. $C_{35}H_{60}O_{14}N_2$. No S, P, or halogens. Equivalent weight about 5000. Negative Molisch, Millon, biuret, Salkowski, Liebermann (steroids and cholesterol), and murexide tests. No color with H_2SO_4 in acetic acid or with I_2. Positive Axenfeld (protein) test (3). Fails to pass a porcelain filter (2). Treatment with methanol containing a few drops of methanolic HCl gives an inactive product, soluble in acetone and chloroform but insoluble in 1 per cent neutral phosphate buffer, $[\alpha]_D^{22} = +32.2° \pm 2.0°$ (c = 0.995 per cent in methanol) (3).

Biological activity: Active against certain fungal plant pathogens including fusaria, gram-positive bacteria, and mycobacteria. Not active on gram-negative bacteria (3).

References:

1. Meredith, C. H. Phytopathology **33:** 403, 1943.
2. Thaysen, A. C. and Butlin, K. B. Nature, London **156:** 781–782, 1945.
3. Arnstein, H. R. V. *et al.* J. Gen. Microbiol. **2:** 111–122, 1948.
4. Mfg. Chemist **22:** 47, 1951.

Mutomycin

Produced by: Streptomyces atroolivaceus var. *mutomycini.*

Method of extraction: The antibiotic is present mainly in the mycelium but can be extracted to a lesser extent from the broth. To extract from the broth, the mycelium is filtered off and the liquid acidified with HCl to pH 3.0. A precipitate forms, which is extracted with acetone (neutralized with NaOH to pH 7.0). The acetone-extract is clarified with 0.4 per cent charcoal and evaporated at 40°C. During concentration, the antibiotic precipitates out. Precipitate dissolved in chloroform at 45°C. After concentration of the chloroform, the antibiotic is precipitated by addition of 5 volumes of petroleum ether. Repeated crystallization from a 65:35 chloroform-benzene mixture. From the mycelium, the antibiotic is extracted with acetone. Acetonic extract purified further as indicated above.

Chemical and physical properties: White powder consisting of needle-shaped crystals; m.p. 141.5–142.0°C. Insoluble in water, ether, 5 per cent sodium hydroxide, sodium carbonate, and hydrochloric acid. Soluble in ethanol and acetone and to a lesser extent in chloroform and benzene. Solubility is increased in organic solvents by a reduction of the pH, but the antibiotic is less stable at acid than at alkaline pH. Molecular weight (Rast), 124. C = 65.5%; H = 9.1%; O = 25.5%. Suggested empirical formula: $C_7H_{11-12}O_2$. No characteristic light absorption in the ultraviolet range.

Biological activity: Active against respiratory-deficient mutants of staphylococci. Not active against other bacteria. Slightly inhibits Ehrlich carcinoma in mice.

Toxicity: Mice tolerate single oral or subcutaneous injection of 0.5 to 1.0 gm per kg.

Reference: 1. Gause, G. F. Antibiotiki **4**(3): 20–23, 1959.

Mycelin

Produced by: Streptomyces roseoflavus (1), and possibly by *S. fradiae* (dextromycin-producer) and *S. diastatochromogenes* (3).

Method of extraction: Mycelium extracted with methanol, ethanol, or acetone. Methanol-extract concentrated *in vacuo*. Ethanol and $Ba(OH)_2$ added to precipitate impurities. Ba^{++} removed with CO_2. Alcohol layer concentrated to precipitate mycelin. Chromatographed on alumina from acetone (1).

Chemical and physical properties: Prisms. Blackens at 260°C and decomposes at 263°C. Soluble in chloroform, butanol, methanol, ethanol, amyl alcohol, acetone, and benzene. Insoluble in water, ether, and petroleum ether. No N or S. Negative Molisch test. Stable to heat and acid, and to alkali in aqueous acetone solution (1).

Biological activity: Active on filamentous fungi;

less active on yeasts. Not active on bacteria (1). More active at alkaline than acid pH. Somewhat active topically against dermal *Trichophyton purpureum* infections in guinea pigs (2).

Toxicity: Reputedly too toxic for systemic use (2).

References:

1. Aiso, K. *et al.* J. Antibiotics (Japan) **5:** 217–219, 1952.
2. Aiso, K. *et al.* J. Antibiotics (Japan) **5:** 488–491, 1952.
3. Igarashi, S. *et al.* J. Antibiotics (Japan) **9A:** 226, 1956.

Mycelin-IMO

Produced by: Streptomyces sp. resembling *S. diastatochromogenes.*

Method of extraction: Present in both the mycelium and culture-filtrate. Isolated by absorption on alumina or activated charcoal, and elution with organic solvents.

Chemical and physical properties: Hexaene. Yellow crystals. Decomposes at 214–222°C. Soluble in acetone, alcohols, butyl acetate, and chloroform. Insoluble in water. Ultraviolet absorption spectrum maxima (in methanol) at 243, 294 (320), 335, 355, and 373 mμ. Infrared spectrum given in reference 1. $[\alpha]_D^{21} = +70.0° \pm 2.0°$ (c = 1 per cent in 1,4-dioxane). Dark green color in concentrated H_2SO_4. Stable substance. Molecular weight, 345. C = 71.29%; H = 5.96%; N = 11.31%. No S or halogens.

Biological activity: Active on fungi and yeasts at 0.5 to 5.0 μg per ml.

Toxicity: LD_{50} (mice) 1.5 mg per kg intraperitoneally.

Reference: 1. Ogata, K. *et al.* Japanese Patent 5,898, 1957.

Mycetin

Produced by: Streptomyces violaceus.

Method of extraction: Extraction of dried, pulverized agar culture with a mixture of equal parts of ethanol and ethylene dichloride (the latter can be replaced by chloroform or benzene). Solvent evaporated and substance dissolved in ethanol.

Chemical and physical properties: Intensely violet in color, although uncertain whether active substance is also colored. Thermostable.

Biological activity: Active against gram-positive bacteria, including micrococci and streptococci, corynebacteria, and spore-formers; also active against mycobacteria. Proteins and pus depress activity. Very limited, if any, activity *in vivo.*

References:

1. Krassilnikov, N. A. and Koreniako, A. I. Mikrobiologiya **8:** 673, 1939; **14:** 80–85, 1945.
2. Fainshmidt, O. I. and Koreniako, A. I. Biokhimiya **9:** 147–153, 1944.

Mycolutein

Produced by: Streptomyces sp.

Method of extraction: Extraction of the mycelium with methanol, concentration of the extract *in vacuo* to one tenth its original volume. On standing at 4°C a precipitate forms, which is exhaustively extracted with warm chloroform. After concentration of the chloroform-extract, addition of petroleum ether results in the formation of a light yellow precipitate. This precipitate is then dissolved in a minimal amount of boiling methanol. On cooling, crystals are formed. Recrystallization from methanol and benzene.

Chemical and physical properties: Bright yellow tabular crystals; m.p. 157–158°C. Absorption of light at 254 mμ ($E_{1cm}^{1\%}$ 680) and at 345 mμ ($E_{1cm}^{1\%}$ 400) in methanolic solution. $[\alpha]_D^{25} = +54°$ (c = 1 per cent in chloroform). Countercurrent distribution (39 transfers) shows the substance to be homogeneous. Soluble in chloroform, acetone, lower alcohols, benzene, dioxane, pyridine, and glacial acetic acid. Soluble to a limited extent in ethyl acetate, carbon tetrachloride, diethyl ether, and petroleum ether. Insoluble in water at all pH values. Decomposed by aqueous sodium hydroxide; dissolves in concentrated sulfuric acid, giving an olive color which turns rapidly to dark red. Stable in boiling methanol for at least 10 minutes in the pH range of 5.1 to 8.2, but rapid decomposition occurs at higher and lower pH values. Decolorizes a solution of potassium permanganate in acetone and a solution of bromine in methanol in the cold. Positive test for aromatic nucleus with anhydrous aluminum chloride and chloroform. $FeCl_3$ test negative. Infrared spectrum given in original paper (1). C = 66.45%; H = 5.95%; N = 3.51%. Freezing-point depression indicates a molecular weight of 417 in benzene and 495 in 1,4-dioxane. Tentative empirical formula: $C_{22}H_{24}NO_6$.

Biological activity: Mycolutein inhibits the growth of *Candida* species, *Cryptococcus neoformans, Trichophyton* species, and *Microsporum gypseum* at the level of 0.2 to 12.5 μg per ml after 48 hours of incubation at 28°C on a medium containing no sugar. The fungi gradually grow at higher concentrations of the antibiotic upon prolonged incubation. The addition of sugars to the

test agar prevents the action of the antibiotic. No activity against bacteria.

Toxicity: Mice are not killed by intraperitoneal administration of 5 mg per kg; 25 mg per kg by the same route kill mice.

Reference: 1. Schmitz, H. and Woodside, R. Antibiotics & Chemotherapy **5:** 652–657, 1955.

Mycomycetin

Produced by: Streptomyces arenae (gray-spored) (1).

Method of extraction: Antibiotic present mostly in mycelium. Extraction may be carried out by: I. Organic solvents, including methanol, acetone, butanol, or t-butanol, at pH 4.0 to 10.0. Extract concentrated to a watery residue, and residue extracted with chloroform at pH 2.0 to 10.0. Chloroform chromatographed on (a) silica gel, and antibiotic eluted with chloroform containing increasing amounts of methanol; or (b) Darco G-60, and eluted with methanol (80 per cent)-chloroform (20 per cent) containing 0.1 *N* HCl. II. Extraction with 50 to 80 per cent ethanol. Extract evaporated to an aqueous residue and extracted with butanol at pH 2 to 4. Re-extraction into water at pH 10 (1).

Chemical and physical properties: Negatively charged acidic substance. Countercurrent distribution studies (pH 7.0; water-butanol) indicate presence of at least two substances, one more soluble in water, the other in butanol. Soluble in dilute NaOH, methanol, acetone, n-butanol, and t-butanol. Alkali-metal salts soluble; precipitate on acidification. Cu, Ca, Mg, and Pb salts insoluble. Positive anthrone test. Negative biuret test. Molecular weight, about 10,000. Nondiffusible; nondialyzable. Ultraviolet absorption spectrum maximum at 260 mμ ($E^{1\%}_{1cm}$ 70). Infrared spectrum given in reference 1. Darkens at about 170°C; chars a little above 200°C. Contains C, H, N, and <1 per cent S; no P. Precipitates with lead acetate and may be regenerated on addition of H_2S or H_2SO_4 in acetone, methanol, or n-butanol (1).

Biological activity: Active mainly on mycobacteria. Moderately active on gram-positive bacteria. Active on streptomycin- and streptothricin-resistant *Staph. aureus*. Not active on gram-negative bacteria or *C. albicans* (1). No *in vivo* activity on tuberculosis in guinea pigs (2).

References:

1. Grundy, W. E. *et al.* Canadian Patent 515,162, August 2, 1955.

2. Grundy, W. E. Personal communication, 1958.

Mycomycin

Produced by: Nocardia acidophilus (10).

Method of extraction: Cooled broth-filtrate extracted at pH 2.5 with hexane in a Podbielniak extractor. Re-extracted into cold 0.5 per cent pH 7.5 sodium phosphate buffer. Buffer stirred with cold methylene chloride, then adjusted to pH 2.0. Extract dehydrated, decolorized, and crystallized from the treated extract on cooling with dry ice-acetone. Recrystallized from methylene chloride or acetone (8). Can also be extracted from broth with ether or amyl acetate (1).

Chemical and physical properties: Unsaturated carboxylic acid. (−)-3,5,7,8-n-tridecatetraene-10,12-diynoic acid (9). *Free acid:* Colorless needles; m.p. 75°C (decomposes explosively). Soluble in methylene chloride and acetone. Ultraviolet absorption spectrum maxima at 267 mμ (ε = 67,000) and 281 mμ (ε = 61,000) with an inflection at 256 mμ (ε = 35,000) (7, 8). Infrared absorption spectrum given in reference 8. $[\alpha]_D^{25} = -130°$ (c = 0.4 per cent in absolute ethanol). Highly unstable to heat and oxidizing conditions. Half-life of crystals at 27°C is 3 hours. Forms a precipitate with alcoholic silver nitrate, which then darkens at room temperature. Solution in liquid ammonia changes from yellow to green, to blue, to purple, to red. Neutral equivalent, 198 (7, 8). *Na salt:* Relatively stable. Decomposes in acid (2). *Methyl ester:* Crystalline; m.p. 44°C (decomposition). Poorly soluble in water. Biologically active. $C_{13}H_{10}O_2$: C = 78.17%; H = 5.36%; C—CH_3 = 0.48%. Structure (7) given in Chapter 6. A relatively stable, biologically active isomer of mycomycin, isomycomycin, has been prepared by treating a dilute aqueous solution of mycomycin with dilute NaOH (1.0 *N*) at room temperature, then treating the resulting precipitate with HCl at pH 2.0. *Isomycomycin:* 3,5-Tridecadiene-7,9,11-triynoic acid (10). Long white needles. Decomposes slowly when heated above 100°C. Soluble in ether, ethanol, dioxane, ethyl acetate, and glacial acetic acid. Slightly soluble in benzene, chloroform, and carbon disulfide. Insoluble in hexane and petroleum ether. Ultraviolet absorption spectrum maxima at 260 and 270 mμ with weak maxima at 290, 307, 327, and 348 mμ. Infrared data given in reference 10. $C_{13}H_{10}O_2$. Possible structural formula given in Chapter 6.

Biological activity: Active on gram-positive and gram-negative bacteria, mycobacteria, and fungi

(2), but not on *Ps. aeruginosa* (4). Early reports of activity against *Mycobacterium tuberculosis* H37Rv in mice and hamsters (3) were not supported by further work with highly purified preparations (6). *Isomycomycin:* Active on mycobacteria (10).

Toxicity: Mice tolerate 25 mg per kg intravenously (4, 8). Guinea pigs tolerate subcutaneously administered mycomycin very poorly, but 2.5 mg per day for 35 days is nontoxic (5).

References:

1. Johnson, E. A. and Burdon, K. L. J. Bacteriol. **54:** 281, 1947.
2. Johnson, E. A. Bacteriol. Proc. 68–69, 1949.
3. Hobby, G. 8th Veterans Admin. Streptomycin Conf. 295–298, 1949.
4. Jenkins, D. E. 9th Veterans Admin. Streptomycin Conf. 179–186, 1950.
5. Jenkins, D. E. 10th Veterans Admin. Conf. Chemotherapy Tuberc. 286–287, 1951.
6. Jenkins, D. E. 11th Veterans Admin. Conf. Chemotherapy Tuberc. 309–310, 1952.
7. Celmer, W. D. and Solomons, I. A. J. Am. Chem. Soc. **74:** 1870–1871, 1952.
8. Celmer, W. D. and Solomons, I. A. J. Am. Chem. Soc. **74:** 2245–2248, 1952.
9. Celmer, W. D. and Solomons, I. A. J. Am. Chem. Soc. **75:** 1372–1376, 1953.
10. Celmer, W. D. U. S. Patent 2,703,328, March 1, 1955.

Mycorhodin

Produced by: Streptomyces sp.

Method of extraction: Extracted from broth-filtrate with chloroform, methyl isobutyl ketone, or n-butanol, at pH 4.0 to 8.0. The mycelium-filter-aid cake is eluted with methanol to recover a minor portion of active component. Concentration of the chloroform-extract precipitates a white, antibiotically inactive substance mixed with red crystals of mycorhodin. Crystallization from methanol or acetone gives the inactive substance as a precipitate. Mycorhodin recovered from the mother liquors on addition of petroleum ether. Recrystallized from hot methanol with water or warm chloroform-ether (1:1). Purified by chromatography on Super-Cel from chloroform-ether, and by countercurrent distribution in a methanol-water-chloroform-carbon tetrachloride (3:1:1.5:4) system or methanol-methyl isobutyl ketone-water (1:1:1).

Chemical and physical properties: Indicator substance. Bright red needles; m.p. 200–202°C (decomposition, corrected). Soluble in aqueous methanol. Ultraviolet absorption spectrum maxima at 258 mμ ($E^{1\%}_{1cm}$ 426), 420 mμ ($E^{1\%}_{1cm}$ 85), and 471 mμ ($E^{1\%}_{1cm}$ 87) (95 per cent ethanol); or at 258 mμ ($E^{1\%}_{1cm}$ 563), 410 mμ ($E^{1\%}_{1cm}$ 79), and 461 mμ ($E^{1\%}_{1cm}$ 82) (95 per cent ethanol-0.1 *N* HCl); or at 595 mμ ($E^{1\%}_{1cm}$ 136) (95 per cent ethanol-0.1 *N* NaOH). Infrared spectrum data given in reference 1. In aqueous methanol, red-orange color changes to purple at pH 8.10, and to deep blue at higher pH values. Dark red-purple color in concentrated H_2SO_4 and alcoholic $FeCl_3$. Brown color in $KMnO_4$. Decolorized by H_2O_2 at alkaline but not at neutral pH. A solution of the antibiotic lost both indicator and antibiotic properties when kept for 12 hours at room temperature, but was stable under these conditions at 6°C. Very stable to acid. Contains no amino acids. Hydrolysis products include reducing sugars. C = 58.7%; H = 5.2%; N = 2.1%. Molecular weight, 635 to 698. *Acetyl derivative:* Yellow crystals. Inferior antibiotic activity. *Dipotassium salt:* Amorphous, blue salt. Water-soluble. Stable.

Biological activity: Active on gram-positive bacteria and mycobacteria, but not on gram-negative bacteria. Active in protecting and curing mice with infections of *D. pneumoniae* and *Staph. aureus.*

Toxicity: LD_{50} (mice) 170 mg per kg intraperitoneally, about 500 mg per kg intramuscularly, and >1250 mg per kg orally. Produces severe delayed toxicity symptoms, including blood abnormalities, in dogs in chronic toxicity studies.

Reference: 1. Misiek, M. *et al.* Antibiotics & Chemotherapy **9:** 280–285, 1959.

Mycospocidin

Produced by: Streptomyces bobiliae.

Method of extraction: Broth extracted with butanol; mycelium with methanol-pyridine (3:1). Methanol-pyridine evaporated and extracted with butanol. Combined butanol-extracts washed with water at pH 8.0 and pH 2.0, and then evaporated to about 1/20 volume. Addition of acetone to concentrate precipitates crude mycospocidin. Purification by chromatographing a pyridine solution on alumina suspended in methanol-benzene, washing with methanol-benzene (1:1), and eluting with methanol-pyridine (1:1). Further purification by countercurrent distribution (ethyl acetate-pyridine-water, 5:1:5).

Chemical and physical properties: White crystalline powder; m.p. 233–234°C (decomposition). $[\alpha]^{26}_{D} = +56°$ (1.0 per cent in pyridine). Soluble in pyridine and aqueous NaOH. Sparingly soluble in methanol and ethanol. Insoluble in acetone, ethyl acetate, benzene, chloroform, carbon tetra-

chloride, ether, and petroleum ether. Ultraviolet absorption spectrum maxima at 215 mμ ($E^{1\%}_{1cm}$ 215) and 257 to 258 mμ ($E^{1\%}_{1cm}$ 89) (methanol). Infrared spectrum given in reference 1. Positive diazo reaction. Negative ninhydrin, biuret, Tollen, $FeCl_3$, and Fehling tests. Stable to heating for 10 minutes at 100°C at pH 2.0 to 9.0. Rf values of 0.53 (water-saturated butanol) and 0.86 (phenol-water, 8:2). C = 54.26%; H = 7.57%; N = 6.43%. $C_{20}H_{32}N_2O_9$. Acid hydrolysis products include two ninhydrin-positive products, one of which may be glycine.

Biological activity: Active against gram-positive bacteria and mycobacteria; moderately active on fungi. Not active on gram-negative bacteria. No activity on the ascitic form of Ehrlich carcinoma in mice.

Toxicity: LD_{50} (mice) 1 to 2 mg per kg intraperitoneally.

Reference: 1. Nakamura, S. *et al.* J. Antibiotics (Japan) **10A:** 248–253, 1957.

Mycothricins

Produced by: Streptomyces lavendulae strains (2), *Streptomyces* sp. (4).

Remarks: Two mycothricin complexes (A and B) were described, each from a different *S. lavendulae* strain and each varying in the number of components present. Complex B contains streptothricin and a component also present in "streptothricin VI" (see streptothricin-like antibiotics). It also contains a component (IV) apparently unique to the mycothricin complex (2, 4).

Method of extraction: I. Culture-broth treated with Darco G-60 at pH 2.5. Filtrate adjusted to pH 7.5 and adsorbed onto fresh Darco. Elution with 75 per cent ethanol. Eluate adjusted to pH 7.0 with IR-45 (OH^- form), concentrated *in vacuo*, and precipitated with acetone. Freeze dried from an aqueous solution (2). II. Broth acidified to pH 3 with acetic acid and treated with oxalic acid to remove the contaminating Ca^{++} and Mg^{++}. Filtered. Chromatographed at pH 6.0 on IRC-50 buffered with ammonium acetate at pH 6.5. Eluted with 10 per cent acetic acid. Active fractions treated by passing through a column of IR-45 (OH^- phase) to reduce pH to 4.0, lyophilized, and taken up in water. Evaporated to dryness *in vacuo*. Solid taken up in absolute methanol, then precipitated with acetone. Chromatographed on Darco G-60-Super-Cel (2:1) mixture from 0.5 *N* HCl. Developed with distilled water to give Fractions 1 and 2, then with 70 per cent ethanol in 0.1 *N* HCl to give Fraction 3. Purified by salt conversion (helianthate to hydrochloride). Component IV purified from Fraction 3 by adjusting to pH 4.0 by passing through an IR-45 (OH^- phase) column, concentration *in vacuo*, and lyophilization. Purified by salt conversion (helianthate → hydrochloride) (4).

Chemical and physical properties: Complexes A and B: Peptide-like, strong organic bases. Yellow-brown powder. *Hydrochlorides:* Colorless, water-soluble substances. Heat-stable. *Bases:* Soluble in water. Slightly soluble in methanol and ethanol. Insoluble in n-butanol, benzene, ether, petroleum ether, ethyl acetate, chloroform, and acetone. Positive ninhydrin, Pauly, and biuret tests. Negative or inconclusive Fehling, Tollen, Molisch, maltol, Sakaguchi, Millon, and Hopkins-Cole tests. More stable to heat in acid and neutral than in alkaline solutions. Destroyed by autoclaving at 15 pounds for 10 minutes. Complex A more stable than Complex B. Acid hydrolysates contain five to seven ninhydrin-positive products, including β-lysine, streptolidine, and serine. *Component IV:* $[\alpha]_D^{25} = -1.1°$ (c = 0.526 per cent in water). Positive Benedict test after heating for 10 minutes. Weakly positive anthrone test. *Helianthate:* Purple crystals; m.p. 200–205°C. *Hydrochloride:* White, amorphous solid (1, 4).

Biological activity: Complex A slightly more active than B. Both are active against gram-positive and gram-negative bacteria, fungi, and yeasts (2). Active on the nematode *Rhabditis briggsae* (5). Reduction, by seed treatment, of *Helminthosporium* sp., *Fusarium* sp., and bacteria on wheat seeds (3). Complexes are more active than streptothricin on *B. mycoides*, but less so on *Ps. fluorescens*. Mycothricins are more active on fungi and yeasts than all other streptothricins except pleocidin (1, 2). *Component IV:* three to ten times more active than the complex on bacteria and 30 times more active on *C. tropicalis* and *Sacch. cerevisiae* (4).

Toxicity: LD_{50} (mice) 65 to 130 mg per kg (Complex A) and 32.5 mg per kg (Complex B) subcutaneously. Delayed toxicity typical of the streptothricin group. Not irritating to the rabbit conjunctiva at levels which give reddening and pus with streptothricin (2). Nontoxic to tomato, cucumber, and wheat at 5000 ppm (seeds), 2500 ppm (foliage spray), or 500 ppm (tomato and cucumber in liquid culture) (3).

References:

1. Schaffner, C. P. *et al.* Resumés 2nd Congr. Intern. Biochem., Brussels 95, 1955.
2. Rangaswami, G. *et al.* Antibiotics & Chemtherapy **6:** 675–683, 1956.
3. Rangaswami, G. Plant Disease Reptr. **40:** 483–487, 1956.

4. Horowitz, M. I. Thesis, Rutgers University, 1957.
5. Tiner, J. D. and Rangaswami, G. Proc. Helminthol. Soc. Wash. D. C. **24:** 70–71, 1957.

Mycoticin

Produced by: Streptomyces ruber.

Synonym: Similar to flavofungin.

Method of extraction: Yellow pigment extracted from mycelium with warm 95 per cent ethanol. Crystallized by adding water to this solution. Can also be extracted with methanol and crystallized from an acetone-water mixture.

Chemical and physical properties: Yellow crystals that fluoresce in ultraviolet light. Inactivated rapidly by ultraviolet light, losing fluorescence. Storage in dark *in vacuo* prolongs activity. No N, S, or halogens. Negative bromine and potassium permanganate tests; positive test for ketones with dinitrophenylhydrazine sulfate. Reduces ammoniacal silver nitrate and Fehling's solution. Soluble in lower alcohols, propylene and diethylene glycol, sulfuric and phosphoric acids; partly soluble in water, benzene, and acetone; insoluble in ether, petroleum ether, xylene, and chloroform. Dialyzable. Probable formula: $C_{18}H_{30}O_5$: C = 65.90%; H = 9.43%. Ultraviolet absorption maxima in methanol at 363, 263, and 210 mμ. The 363 mμ band increases in height with loss of activity, brought about by heating with alcoholic 0.6 *N* sulfuric acid, aqueous alcoholic 6 *N* hydrochloric acid, and aqueous alcoholic 10 per cent potassium hydroxide on a steam bath for 2 hours; not affected by heating with aqueous acetic acid or aqueous alcoholic sodium bicarbonate. Maximal activity at alkaline pH; little or none at pH 2 to 5; slight at pH 6 to 7. Antifungal activity unaffected by heating at 100°C for 10 minutes at pH 6. Infrared spectrum in mineral oil gives strong hydroxyl band at 3.1 μ, medium band at 5.94, 6.2, and 6.34 μ. In pyridine, mycoticin gives broad bands at 3.2 to 3.6 and 5.9 to 6.4 μ. A tetraacetyl derivative is formed by adding acetic anhydride and pyridine at room temperature. Melting point of acetate: 140–141°C. C = 63.05 to 63.71%; H = 7.62 to 7.93%. Ultraviolet absorption maxima for acetate at 361 and 260 mμ. Infrared spectrum of acetate in chloroform shows bands at 3.45, 5.75 to 5.78, 6.13, 6.3, and 7.95 mμ. Hydrogenation products of antibiotic and acetate are colorless, do not fluoresce, have no ultraviolet maximum and no antifungal activity.

Biological activity: Active principally on yeasts and some filamentous fungi. Not active on bacteria or protozoa.

Toxicity: LD_{50} (mice) 10 to 20 mg per kg intraperitoneally. No cutaneous sensitivity (humans) reaction noted in 30 persons tested.

Reference: 1. Burke, R. C. *et al.* J. Invest. Dermatol. **23:** 163–168, 1954.

Myxoviromycin

Produced by: Streptomyces sp. resembling *S. albus* (4).

Remarks: This culture produces seven antibiotics, including toyocamycin and actinoflocin (2).

Method of extraction: Broth adjusted to pH 4.0 and filtered. Filtrate adjusted to pH 7.6 and active fraction adsorbed on IRC-50 (H^+ form). Eluted with 0.2 *N* HCl. Active fractions collected and concentrated *in vacuo* at pH 4.0. Treated with butanol at pH 8.0 to remove other antibiotics. Aqueous layer concentrated to dryness at pH 4.0. Extracted with acidic methanol and precipitated with acetone. Purification by chromatography on a carbon-diatomaceous earth mixture (6:4) at pH 7.0 from water. Developed with 2 per cent aqueous acetone (1).

Chemical and physical properties: Basic substance. *Reineckate:* Crystalline platelets; m.p. 184–189°C (1) or 206–209°C (3). Soluble in water and methanol. Insoluble in most other organic solvents. Infrared spectrum given in reference 1. C = 24.53%; H = 4.22%; N = 24.55%; S = 29.1%; Cr = 13.02%. *Hydrochloride:* m.p. 183–185°C (4). Conflicting reports on ninhydrin and biuret tests (1, 3). Yellow color with Pauly test (3). Negative Sakaguchi, Fehling, Molisch, Millon, $FeCl_3$, maltol, and Elson-Morgan tests. Only end-absorption of ultraviolet light. $[\alpha]_D^{23}$ = +2.8° (c = 1.06 per cent in water). Stable at pH 2.0 to 5.0, but not at alkaline pH. Hydrolysis products include β-alanine and methionine or valine (1, 3).

Biological activity: Active on influenza A virus in tissue culture, mice, and eggs. Possibly inhibits intracellular growth of the virus. Active in contact tests against influenza A (FM-1), influenza B (Lee), Newcastle disease virus, and the HVJ (hemagglutinating virus of Japan or Sendai virus). No activity on bacteria, fungi, or yeasts, except some mild activity on *Sarcina lutea* and *B. subtilis* (1, 2, 5, 6–8).

Toxicity: LD_{50} (mice) >200 mg per kg (1) or 37.5 to 50 mg per kg (3) intraperitoneally.

References:

1. Kuroya, M. *et al.* Japan. J. Microbiol. **1:** 85–90, 1957.
2. Katagiri, K. *et al.* Shionogi Kenkyusho Nempo **7:** 715–723, 1957.
3. Kobayashi, N. Chemotherapy **5:** 149–150, 1957.

4. Sato, K. and Katagiri, K. Chemotherapy **5:** 182–183, 1957.
5. Miyakawa, T. *et al.* Japan. J. Microbiol. **2:** 53–62, 1958.
6. Hinuma, Y. *et al.* Japan. J. Microbiol. **2:** 63–68, 1958.
7. Hinuma, Y. *et al.* Japan. J. Microbiol. **2:** 117–125, 1958.
8. Anzai, O. Virus (Osaka) **8:** 174–181, 1958.

Narbomycin

Produced by: Streptomyces narbonensis.

Method of extraction: Extraction of culture fluid at pH 8.5 with ethyl acetate. Transferred into dilute acetic acid. Re-extraction with ethyl acetate after neutralization with sodium carbonate. Crude bases are chromatographed over alumina. Crystallization of active fractions from ether-petroleum ether.

Chemical and physical properties: Colorless crystals; m.p. 113.5–115°C. $[\alpha]_D$ = +68.5° (c = 1.35 per cent in chloroform). pK* about 7.8 in 80 per cent methyl Cellosolve (base). $C_{28}H_{47}O_7N$. Belongs probably to the macrolide group of antibiotics. Maximal light absorption at 225 mμ (log ε = 4.06) and 286 mμ (log ε = 2.23). Infrared spectrum given in the original paper (1). Narbomycin yields desosamine on acid hydrolysis.

Biological activity: Active against gram-positive bacteria. Cross-resistance with picromycin.

Toxicity: LD_0 (mice) 500 mg per kg subcutaneously.

References:

1. Corbaz, R. *et al.* Helv. Chim. Acta **38:** 935–942, 1955.
2. Anliker, R. *et al.* Helv. Chim. Acta **39:** 1785–1790, 1956.

Neocide

Produced by: Probably an actinomycete.

Method of extraction: Dialysis and freeze drying.

Chemical and physical properties: Stable polypeptide containing cysteine, arginine, lysine, glycine, cystine, glutamic acid, aspartic acid, serine, alanine, and proline.

Biological activity: Antitumor and antibacterial activity. Active in mice on Ehrlich ascites carcinoma and in rats on sarcoma 45. Causes reduction of number of mitotic figures and necrosis in cancer cells.

Toxicity: Said to be nontoxic.

Reference: 1. Derkach, V. S. Antibiotiki **2**(5): 40–44, 1957.

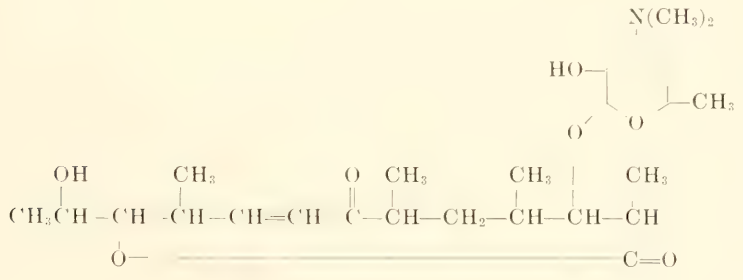
Neomethymycin

Produced by: Streptomyces sp. This strain also produces methymycin (1).

Remarks: Differs chemically from methymycin only in location of one hydroxyl group (2).

Method of extraction: Chromatography of the mother liquors during the isolation of methymycin on alumina (deactivated with 10 per cent aqueous acetic acid). Ether used as solvent and 0.5 to 1.0 per cent methanolic ether as developer. Methymycin is eluted first, followed by neomethymycin. Crystallized from ether-hexane (2).

Chemical and physical properties: Macrolide; m.p. 156–158°C. Isomeric with methymycin and picromycin. $[\alpha]_D$ = +93° ($CHCl_3$). Ultraviolet absorption spectrum maximum (ethanol) at 227.5 mμ (log ε = 4.10). $C_{23}H_{43}O_7N$: C = 63.75%; H = 9.04%; N = 3.07%; N—$(CH_3)_2$ = 5.9%; C—CH_3 = 16.76%; —OCH_3 = 0.0%. Neutral equivalent (perchloric acid titration), 472. Acid hydrolysis (HCl) yields desosamine HCl. (This is also a hydrolysis product of picromycin, erythromycin, narbomycin, and methymycin.) *Methylene solvate:* Large hexagonal plates; m.p. 154–156°C with a gas given off at 135–140°C. $[\alpha]_D$ = +66° (ethanol). *Acetone solvate:* Long crystals; m.p. 156–158°C. Complete structure of neomethymycin (1, 2):



Biological activity: Virtually identical to that of methymycin, except that neomethymycin is 4 times more active on *B. subtilis* (2).

References:

1. Djerassi, C. and Halpern, D. J. Am. Chem. Soc. **79:** 2022–2023, 1957.
2. Djerassi, C. and Halpern, D. Tetrahedron **3:** 255–268, 1958.

Neomycin

Produced by: Streptomyces fradiae, S. albogriseolus, Streptomyces sp. (2).

Synonyms: Similar to or closely related to streptothricins BI and BII, flavomycin, framycetin, catenulin, dextromycin, colimycin (7), miserin (6), mycerin, and antibiotic 956 (5).

Remarks: Among the various preparations comprising neomycin fractions, framycetin occupies a prominent place. Since considerable literature has accumulated concerning this preparation, it is discussed separately.

Method of extraction: I. Clarification of broth at pH 2.0 with charcoal. Adsorption of neomycin at alkaline pH on charcoal; elution with acidic ethanol or acetone. II. Adsorption of neomycin on IRC-50 (resin equilibrated at pH 7 to 8 with alkali); elution with aqueous HCl, H_2SO_4, or ammonia. Freeze dried. III. Alkylbenzene or alkylnaphthalene sulfonic acid added to broth at pH 3 to 5; the resulting sulfonic acid salt of neomycin extracted with butanol. Re-extracted into dilute mineral acid. Neutralization and removal of excess mineral acid with $Ba(OH)_2$ or $Ca(OH)_2$ or with suitable ion exchange resin. Purification (a) by conversion of one salt to another; *i.e.*, neomycin sulfate to the *p*-hydroxyazobenzene-*p'*-sulfonate salt to the purified hydrochloride; (b) by suitable ion exchange resins (Amberlite XE-89 and Dowex 50 X16); (c) by acetone precipitation of ash from an aqueous neomycin solution at pH 10.1 and subsequent acetone precipitation of purified neomycin sulfate from the neutralized filtrate; (d) by carbon chromatography. Separation of neomycins B and C by chromatography on carbon or alumina (2).

Chemical and physical properties: Neomycin is a complex composed of two isomeric entities: neomycin B and neomycin C.

Neomycin B

Free base: Colorless, amorphous solid. $[\alpha]_D^{22}$ = +83° (c = 1 to 2 per cent in 0.2 *N* HCl). Very soluble in water; insoluble in ethanol or methanol. *Sulfate:* Colorless, amorphous solid. $[\alpha]_D^{22}$ = +58° (c = 0.5 per cent in water) or +56°. Same solubility as free base. Base content about 68 per cent. *Reineckate:* Pink platelets from aqueous acetone. Insoluble in water; soluble in hot water and acetone. *N-Acetate:* Colorless needles from aqueous acetone; m.p. 200–205°C (decomposition); softens at 185–190°C. $[\alpha]_D^{22}$ = +62° (c = 0.4 per cent in water). Acetyl content 32 per cent. Biologically inactive; cannot be converted to free neomycin.

Neomycin C

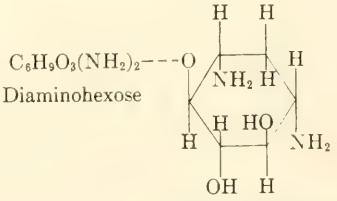
Free base: Colorless amorphous solid. $[\alpha]_D^{22}$ = +121° (c = 1 to 2 per cent in 0.2 *N* H_2SO_4). Very soluble in water; insoluble in ethanol or methanol. *Sulfate:* Colorless, amorphous solid. $[\alpha]_D^{22}$ = +82° (c = 0.5 per cent in water). Same solubility as free base. Base content about 68 per cent. *Helianthate:* Similar to neomycin B. *N-Acetate:* Colorless needles from aqueous acetone; melting point similar to neomycin B N-acetate, but gives 10° depression in mixed melting point determination. $[\alpha]_D^{22}$ = +90° (c = 0.4 per cent in water). Acetyl content 32%. Biologically inactive; cannot be converted to free neomycin.

Neomycin Complex

Basic, white substance. Water-soluble; insoluble in organic solvents. $C_{23}H_{46}N_6O_{13}$. No typical ultraviolet spectrum (2). Infrared spectrum given in reference 1. Positive Molisch, carbazole, and ninhydrin tests. Negative Elson-Morgan, Fehling, and Tollen tests. No acidic, carbonyl, methoxyl, guanidine, or free aldehyde groups. Crude neomycin is stable from pH 2 to 9; highly purified preparations stable to alkali only (1, 2). Acid hydrolysis products include a nonreducing base, neamine (neomycin A), $C_{12}H_{24-26}N_4O_6$, and a methylglycoside moiety.

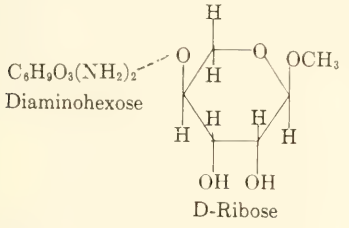
Neamine

Precipitates from alcoholic ammonia as fine needles; m.p. 250–256°C (decomposition). $[\alpha]_D^{22}$ = +123° (c = 0.7 per cent in water). Infrared spectrum given in reference 2. Tentative structure of neamine:

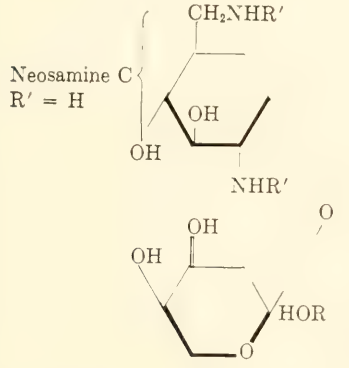


2-Desoxystreptamine

Methylglycosides: These are termed methyl neobiosaminides B and C, and can be differentiated by paper chromatography and specific rotation. Fehling's and Tollen's reagents reduced. Contain primary amino groups. $C_{12}H_{24}N_2O_8$:



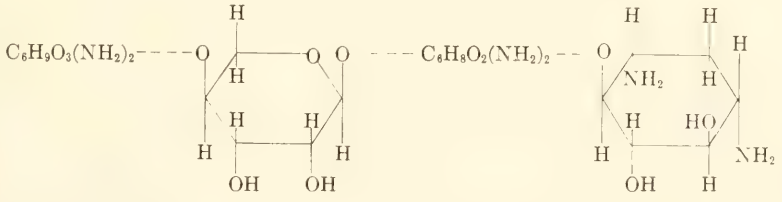
Chemically, neomycins B and C differ only in the diaminohexose (neosamine) portion of the neobiosamine portion of their molecules (1). Neosamines B and C are isomeric (1). The structure of methyl neobiosaminide C, neobiosamine C, and neosamine C (2):



Neobiosamine C: R = R′ = H

Methyl neobiosamide C: R = CH_3; R′ = H

Tentative structure of neomycin:



Biological activity: Active on most gram-positive and gram-negative rods, many gram-positive cocci, mycobacteria, and actinomycetes. Slightly active on certain algae and yeasts. Not active on viruses, protozoa, filamentous fungi, or clostridia. Cross-resistance exists, in certain cases, between neomycin and viomycin, streptothricin, streptomycin, and the mild silver protein Argyrol. The activity of neomycin B is greater than that of C, and there are some qualitative differences between their activities. Neomycin is active *in vivo* against a variety of infections, including those caused by *Neisseria intracellularis, K. pneumoniae, Pasteurella multocida, H. influenzae, V. cholerae,* and *Pr. vulgaris.* It is also effective against salmonellosis, plague, and anthrax, but not promising against tuberculosis or infections with gram-positive cocci, except *Staph. aureus.* Active on rickettsial pox in pigs, and on amebiasis in rats (2). Antifungal activity of endomycin enhanced by the neomycins (4). Neamine has little biological activity, being active mainly against gram-positive bacteria.

Toxicity: Subcutaneous LD_{50} (mice) of neomycins B and C hydrochlorides are 220 and 290 mg per kg, respectively. Intravenous LD_{50} of commercial neomycin as sulfate (mostly neomycin B) is 26.2 to 42.5 mg base per kg. Local toxicity is slight. Nephrotoxic. Causes loss of hearing (2). *Neamine*: LD_{50} (mice) 320 mg per kg intravenously, 1250 mg per kg subcutaneously.

Utilization: Urinary infections refractory to other antibiotics. Infantile diarrhea. Intestinal asepsis. Topical applications (2).

References:

1. Waksman, S. A., ed. Neomycin. Rutgers Univ. Press, New Brunswick, N. J., 1953.
2. Waksman, S. A., ed. Neomycin; its nature and practical applications. The Williams & Wilkins Co., Baltimore, 1958.
3. Rinehart, K. L., Jr. *et al.* J. Am. Chem. Soc. **80:** 6462, 1958.
4. Rinehart, K. L., Jr. and Woo, P. W. K. J. Am. Chem. Soc. **80:** 6463-6464, 1958.
5. Trai Joun-chen *et al.* Antibiotiki 3(2): 27–28, 1958.
6. Kotchetkova, G. V. and Popoba, O. L. Antibiotiki 1(4): 37–40, 1956.
7. Gause, G. F., ed. The antibiotic colimycin and its clinical application. Moscow, 1959.
8. Sokolski, W. T. and Burch, M. R. Antibiotics & Chemotherapy **10:** 157–162, 1960.

Neonocardin

Produced by: Nocardia kuroishi (2).

Method of extraction: Broth containing mycelium heated to 100°C for 20 minutes, then cooled and filtered. Treated with diatomaceous earth at pH 2.0, then adsorbed on activated carbon at pH 5 to 7. Carbon washed with water, methanol, and ether. Elution into 0.04 *N* HCl-methanol. Addition of ether to eluate precipitates neonocardin. Reprecipitated from absolute methanol with ether. Can be extracted from the dried mycelium with distilled water (3).

Chemical and physical properties: Hydrochloride: Yellow-gray or grayish white powder (3). Found to differ from other known antibiotics on paper chromatography (4).

Biological activity: Culture-filtrate active on gram-positive and gram-negative bacteria (1).

Toxicity: Mice tolerate 2 mg intraperitoneally (3).

References:

1. Uesaka, I. J. Antibiotics (Japan) **3:** 27–34, 1950.
2. Uesaka, I. J. Antibiotics (Japan) **5:** 75–79, 1952.
3. Uesaka, I. J. Antibiotics (Japan) **5:** 154–159, 1952.
4. Ueda, S. and Uesaka, I. J. Antibiotics (Japan) **5:** 170–171, 1952.

Netropsin

Produced by: Streptomyces netropsis (2), *Streptomyces* sp. resembling *S. netropsis* (7, 12), *Streptomyces* sp. (6, 13), *S. ambofaciens* (6), *Streptomyces* sp. belonging to the *S. reticuli* group (15), *Streptomyces* sp. (16).

Synonyms: Antibiotic 1A-887 (7), sinanomycin (12), congocidin (11, 13), antibiotic T 1384 (13), antibiotic K 117 (16).

Method of extraction: I. Broth-filtrate stirred with ammonium oxalate at pH 6.5 to precipitate Ca^{++}, then stirred with orange II at pH 5.5 to precipitate the antibiotic. Dye precipitate filtered with Super-Cel, then dye dissociated from the antibiotic by stirring with 80 per cent acetone-20 per cent methanol containing methanolic triethylamine sulfate. Super-Cel-netropsin sulfate mixture washed with acetone-methanol, then extracted with cold distilled water and $BaCl_2$ to give the HCl salt. Adjusted to pH 2.0 with H_2SO_4 to precipitate Ba^{++}, then treatment with IR-4 ion exchange resin to remove excess $SO_4^{=}$. A saturated aqueous solution of the amorphous product gives crystals on standing (2). II. Broth-filtrate adsorbed on IRC-50 cation exchange resin (equilibrated to pH 7.5 with NaOH). Eluted with 0.46 *N* HCl or methanolic HCl. Active fractions adjusted to pH 6.0 and excess Ca^{++} removed as oxalate. Filtrate concentrated *in vacuo*, then cooled to give crystals of antibiotic. Recrystallized from water or methanol. Purification on alumina (3, 11). III. Adsorption from broth-filtrate on acidic clay at pH 7.6 and extraction of mycelium with 80 per cent acetone (pH 5.0). Elution from clay with 80 per cent acetone (pH 2.0). Eluate concentrated *in vacuo*, adjusted to pH 2.0, and lyophilized. Solid extracted with ethanol and precipitated on addition of ether. Purification by chromatography on alumina from ethanol-methanol (3:1) and development with methanol. Precipitated from active fractions with ether (7).

Chemical and physical properties: Unstable, diacidic base. *HCl salt:* Fine needles; m.p. 167–173°C (decomposition) or long, thin, colorless, hydrated prisms exhibiting oblique extinction. Soluble in methanol and ethanol; moderately soluble in water and water-saturated n-butanol. Insoluble in almost all other nonpolar solvents. No optical activity in water. Ultraviolet absorption spectrum maxima at 295 mμ ($E^{1\%}_{1cm}$ 423) and 238 mμ ($E^{1\%}_{1cm}$ 430). Infrared spectrum given in reference 2. Positive Sakaguchi, Dragendorff (N—CH_3), Ehrlich aldehyde (3), Weber-Rose, and Bayer tests. Negative ninhydrin, biuret, Tollen, Fehling, maltol, Molisch, fuchsinaldehyde, murexide, amino antipyridine (phenol), 2,4-dinitrophenylhydrazine, Elson-Morgan, $FeCl_3$, and Hanke-Koessler tests. Inactivated by 0.2 *N* NaOH in <2 hours at 25°C. C = 42.9%; H = 5.78%; N = 28.1%; Cl (total and ionic) = 13.7% (2, 7, 10–12). *Sulfate:* Long colorless needles; m.p. 224–225°C. $C_{18}H_{26}N_{10}O_3 \cdot \frac{1}{2}H_2SO_4$. More soluble in hot than in cold water. Insoluble in common organic solvents. Ultraviolet absorption spectrum maxima at 236 mμ ($E^{1\%}_{1cm}$ 429) and 297 mμ ($E^{1\%}_{1cm}$ 436). Differentiated from streptothricin and streptomycin by paper chromatography (water-saturated n-butanol containing 2 per cent *p*-toluenesulfonic acid). Hydrogenation product is biologically inactive (1, 2, 7, 10). *Picrate:* Sheaves of yellow needles; m.p. 205 or 232°C (decomposition). Browns and sinters at 225°C (1, 7, 10). *Helianthate:* Orange plates; m.p. 215°C (decomposition) (10). Mild alkaline hydrolysis gives either glycocyamidine or guanidinoacetic acid, ammonia, and β-[4-(4-amino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]propionamide (netropsinine). The latter compound was also obtained from congocidin and antibiotic T 1384 (10, 11, 13). Structure of netropsin (13) given in Chapter 6. β-[4-(4-guanidino-

acetamidino-1-methyl-2-pyrrolecarboxamido)-1-methyl-2-pyrrolecarboxamido]propionamide.

Biological activity: Active on gram-positive and gram-negative bacteria, but not on *Ps. aeruginosa*. Very slightly active on *C. albicans* (90 μg per ml) (2), *P. chrysogenum*, and *Piricularia oryzae* (7). Active on *Trichomonas vaginalis* and *T. foetus in vitro* at 12 μg per ml (2, 5). Active on *Chlorella pyrenoidosa* at 10 μg per ml (14). Has antiphage activity (9). Active on clothes moth larvae and the black carpet beetle (1, 2). No cross-resistance with chloramphenicol, streptomycin, or streptothricin (2). Active in mice on vaccinial infections (4). Conflicting reports on anti-influenza activity (4, 12). No activity *in vivo* on feline pneumonitis, western equine encephalomyelitis, or poliomyelitis (4). Moderate inhibition of Mecca lymphosarcoma (mice) and slight inhibition of carcinoma 1025 (mice) and Walker carcinosarcoma 256 (rats) (8). Not active on Ehrlich ascites carcinoma (12).

Toxicity: LD_{50} (mice) 17 mg per kg intravenously, 70 mg per kg subcutaneously, and >300 mg per kg orally (1).

References:

1. Finlay, A. C. *et al.* J. Am. Chem. Soc. **73:** 341–343, 1951.
2. Finlay, A. C. and Sobin, B. A. U. S. Patent 2,586,782, 1952.
3. Despois, R. and Ninet, L. Riass. commun. 6th Congr. Intern. Microbiol. **1:** 162–163, 241–242, 1953.
4. Schabel, F. M., Jr. *et al.* Proc. Soc. Exptl. Biol. Med. **83:** 1–3, 1953.
5. Seneca, H. and Ides, D. Am. J. Trop. Med. Hyg. **2:** 1045–1049, 1953.
6. Pinnert-Sindico, S. Ann. inst. Pasteur **87:** 702–707, 1954.
7. Isono, K. *et al.* J. Antibiotics (Japan) **8A:** 19–21, 1955.
8. Sugiura, K. Cancer Research **3:** (suppl.) 18–27, 1955.
9. Asheshov, I. N. *et al.* Cancer Research **3:** (suppl.) 57–62, 1955.
10. van Tamelen, E. E. *et al.* J. Am. Chem. Soc. **78:** 2157–2159, 1956.
11. Julia, M. and Joseph, N. Compt. rend. **243:** 961–964, 1956.
12. Watanabe, K. J. Antibiotics (Japan) **9A:** 102–107, 1956.
13. Waller, C. W. *et al.* J. Am. Chem. Soc. **79:** 1265–1266, 1957.
14. Tomisek, A. *et al.* Plant Physiol. **32:** 7–10, 1957.
15. Thrum, H. Naturwissenschaften **46:** 87, 1959.
16. Magyar, K. *et al.* Abstr. Commun. Symposium on Antibiotics, Prague 26–27, 1959.

Niger Factor

Produced by: Streptomyces sp. resembling *S. albus*. This culture produces six other antibiotics, including myxoviromycin, toyocamycin, actinoflocin, lutea factor, and two tetraenic antifungal antibiotics (1, 2).

Synonym: Said to be related to cycloheximide (1).

Method of extraction: I. Broth extracted with chloroform at acid pH. Chromatography of extract on a cellulose column. Elution with 20 per cent NH_4Cl. Purified by countercurrent distribution (benzol-pH 7.0 phosphate buffer). Crude powder treated with chloroform and crystallized from amyl acetate on standing (1). II. Adsorbed from broth on IRC-50 (H^+ phase) at pH 7.8. Eluted with aqueous 80 per cent acetone. Eluate extracted with chloroform at pH 5. Proceed as in Step I (2).

Chemical and physical properties: Colorless crystals. Ultraviolet absorption maximum at 258 mμ. Stable over a wide range of pH values (1).

Biological activity: Active on certain fungi and yeasts, such as *Willia anomala*, *Saech. sake*, and *A. niger*. Not active on *C. albicans* or *Trichophyton interdigitale* (1). Antiviral activity against influenza A.

Toxicity: LD_{50} (mice) 160 mg per kg intravenously (1).

References:

1. Sato, K. and Katagiri, K. Chemotherapy **5:** 182–183, 1957.
2. Katagiri, K. *et al.* Shionogi Kenkyusho Nempo **7:** 715–723, 1957.

Nigericin

Produced by: Streptomyces violaceoniger strains and related *Streptomyces* spp. (1, 2).

Method of extraction: Precipitated from broth by acidification to pH 2 to 3. Precipitate dissolved in base. Crystallization as Na salt by warming this solution at pH 8.5 or above. Recrystallized from hot methanol on addition of water. Can also be extracted from broth by n-butanol, butyl acetate, or ethyl ether, or adsorbed on charcoal and eluted with methanol (1).

Chemical and physical properties: Acidic substance ($C_{39}H_{69}O_{11}$). *Na salt:* Colorless needles; m.p. 246–254°C. Free acid and Na salt are slightly soluble in water and soluble in lower alcohols.

Free acid: $C_{39}H_{69}O_{11}$: C = 65.42%; H = 9.90%. *Na salt:* 0—CH_3 = 3.99%.

Biological activity: Active on gram-positive bacteria (0.12 to 0.5 μg per ml), mycobacteria (0.5 to 4.0 μg per ml), *C. albicans* (2 μg per ml), *Trichophyton mentagrophytes* (16 μg per ml). Gram-negative bacteria are resistant to 64 μg per ml. Biological activity inhibited by K^+. Inhibits respiration and phosphate uptake in the presence of several substrates; also inhibits mitochondrial adenosinetriphosphatase. This inhibition is dependent in part on the structural integrity of the mitochondria (3).

Toxicity: LD_{50} (mice) 2.5 mg per kg intraperitoneally.

References:

1. Harned, R. L. *et al.* Antibiotics & Chemotherapy **1:** 594–596, 1951.
2. Benedict, R. G. and Lindenfelser, L. A. Given in Benedict, R. G. Botan. Rev. **19:** 229–320, 1953.
3. Lardy, H. A. *et al.* Biochim. et Biophys. Acta **78:** 587–597, 1958.

Niromycins

Produced by: Streptomyces albus.

Synonyms: Closely related to cycloheximide and fermicidin.

Method of extraction: Extraction of broth at pH 7.4 with butanol. Evaporation of butanol under reduced pressure to syrup; washed with petroleum ether. Resulting crude residue dissolved in water and extracted with ethyl acetate; concentrated *in vacuo* to syrup. Syrup washed with petroleum ether and dried. Residue dissolved in butyl alcohol and passed through an alumina column to remove impurities. Active effluent evaporated. Upon addition of benzene, white crystals appear and are removed. Benzene solution evaporated. Residue washed with petroleum ether and dried. Crude powder dissolved in 20 per cent aqueous acetone and chromatographed on carbon. Elution with progressively drier acetone. Fractions active against *Sacch. sake* combined and the acetone evaporated. Active powder purified by countercurrent distribution (benzene-water, 1:1, 30 transfers). One active substance shows a peak at Tube 6 (niromycin A) and another at Tube 9 (niromycin B). Niromycin A is further purified by repeating distribution, but is unstable and does not crystallize. Niromycin B crystallized from ethyl acetate (1).

Chemical and physical properties: Niromycin A: Hygroscopic, white, amorphous, neutral substance; m.p. 98–105°C. Soluble in water, methanol, ethanol, butanol, ethyl acetate, butyl acetate, acetone, and chloroform. Slightly soluble in benzene and ether. Insoluble in petroleum ether. No specific ultraviolet light absorption. No optical rotation (c = 1 per cent in ethanol). Positive Tollen and 2,4-dinitrophenylhydrazine reactions. Negative ninhydrin, $FeCl_3$, Fehling, Benedict, Molisch, biuret, and permanganate reactions (1). *Niromycin B:* Neutral substance. White hygroscopic crystals; m.p. 47–67°C. Same solubility properties, color reactions, light absorption, and optical rotation as niromycin A. C = 62.57%; H = 7.54%; N = 5.3%. Infrared spectrum given in reference 1. *Semicarbazone:* m.p. 175–176°C. C = 42.06%; H = 7.33%; N = 35.90%. *2,4-Dinitrophenylhydrazone:* m.p. 199–200°C (decomposition). C = 56.29%; H = 5.53%; N = 15.16%. Suggested formula for niromycin B: $C_{14}H_{21}NO_4$ (1).

Biological activity: Both niromycins are active against fungi and viruses, but not against bacteria. Examples of minimal inhibitory concentrations in μg per ml: *Hansenula anomala:* A = 1.5, B = 0.7; *Sacch. cerevisiae:* A = 0.35, B = 0.17; *Sacch. sake:* A = 0.7, B = 0.35; *A. niger:* A = B = >1000. In tissue cultures, niromycin A inhibits Newcastle disease virus at 0.01 μg per ml; nontoxic to chick embryo cells at 6.25 μg per ml. Niromycin B inhibits Newcastle disease virus at 0.036 μg per ml and shows no toxicity at 0.75 μg per ml. Both substances inhibit multiplication of influenza virus in chick embryos (2).

Toxicity: Niromycin A: LD_{50} (mice) 40 to 60 mg per kg intravenously. *Niromycin B:* LD_{50} (mice) 48 mg per kg, (rats) 1.8 mg per kg intravenously.

References:

1. Osato, T. *et al.* J. Antibiotics (Japan) **13A:** 110–113, 1960.
2. Osato, T. *et al.* J. Antibiotics (Japan) **13A:** 97–109, 1960.

Nitrosporin

Produced by: Streptomyces nitrosporeus (1).

Method of extraction: I. Adsorption on charcoal or cation exchange resin and elution with acidic acetone or dilute HCl. II. Broth extraction by ethyl ether, butyl or ethyl acetate at pH 7.0. Reextracted into water at pH 2.0. Back-extracted into ethyl acetate at pH 7.0 and extract concentrated *in vacuo*. Chromatographed on alumina, developed with ethyl acetate. Fractions pooled and lyophilized. Solid taken up in water, warmed, filtered, then cooled to precipitate crystals (1).

Chemical and physical properties: Basic white crystalline substance. Browns at 115–120°C; m.p. 130–140°C. Soluble in ethanol and ethyl acetate.

Slightly soluble in ether, benzene, ethylene dichloride, and acidic water. Insoluble in water. Unstable to, and turning brown on, exposure to air. Ultraviolet absorption spectrum maxima at 250 and 320 mμ. Negative $FeCl_3$, ninhydrin, biuret, Molisch, and Fehling tests. $C_{20}H_{26}N_2O_6$: C = 60.34%; H = 6.41%; N = 7.88%. No S or halogen (1).

Biological activity: Active on gram-positive bacteria; less so on gram-negative bacteria. Not active on *B. anthracis*. Not active on fungi or mycobacteria (1).

Toxicity: LD_{50} (mice) 16 mg per kg intravenously (1).

Reference: 1. Umezawa, H. and Takeuchi, T. J. Antibiotics (Japan) **5:** 270–273, 1952.

Nocardamin

Produced by: Nocardia sp. similar to *N. flavescens* (1).

Method of extraction: Mycelium-containing culture-broth extracted with butanol. Extract concentrated to dryness *in vacuo*. Residue washed and pulverized with ether, filtered, then taken up in hot water. Crystallizes on cooling (1). Recrystallized from water-saturated butanol or hot water (2).

Chemical and physical properties: Reddish needles (1,2); m.p. 183–184°C. Soluble in boiling methanol and dilute NaOH. Slightly soluble in hot water (1). Optically inactive. Positive ammoniacal silver nitrate and Fehling tests (2). Hygroscopic (1). Gives a red-brown color with $FeCl_3$ (aqueous or alcoholic) and a green color with copper chloride (1). *Monoacetate*: Fine needles; m.p. 118°C. $C_{11}H_{16}O_6N_2$: C = 54.84%; H = 6.90%; N = 11.34%; $COCH_3$ = 17.57% (2). *Base*: $C_9H_{14}O_3N_2$ (2). Structure of nocardamin given in Chapter 6.

Biological activity: Active on mycobacteria. Not active on bacteria or fungi (2). Inactivated by serum (1).

References:

1. Stoll, A. *et al.* Schweiz. Z. Pathol. u Bakteriol. **14:** 225–233, 1951.
2. Stoll, A. *et al.* Helv. Chim. Acta **34:** 862–873, 1951.

Nocardianin

Produced by: Nocardia sp. (1).

Method of extraction: Filtered broth extracted with ether. Extract dehydrated and evaporated to dryness. Residue dissolved in chloroform-ether (50:50) or benzene and chromatographed on alumina. A reddish material eluted with chloroform-ether or chloroform; recrystallized from methanol (1).

Chemical and physical properties: Weak base (2). $C_{65-67}H_{96-104}O_{15}N_{18}$: C = 57.25%; H = 7.25%; N = 18.3%. No S, P, or halogens. Red prisms; m.p. 228–235°C (decomposition). Soluble in chloroform, glacial acetic acid, and pyridine; moderately soluble in acetone, methanol, and dilute acids; sparingly soluble in water and ether; insoluble in petroleum ether, carbon disulfide, and carbon tetrachloride. $[\alpha]_D^{25} = -223°$ (c = 0.3 per cent in methanol). Ultraviolet absorption spectrum maximum at 440 mμ ($\log \epsilon = 4.52$) (methanol). Infrared spectrum given in reference 1. Negative xanthine, Liebermann, Schiff, Ehrlich, biuret, and hydroxamic acid tests. Acid hydrolysates give negative ninhydrin and Ehrlich tests, but a methanolic solution of alkali fusion products to which concentrated HCl has been added gives a positive Ehrlich test, indicating possible presence of pyrrole nuclei.

Biological activity: Active on gram-positive bacteria; not active against gram-negative bacteria or mycobacteria.

References:

1. Bick, I. R. *et al.* Antibiotics & Chemotherapy **2:** 255–258, 1952.
2. Cram, D. J. Given in Benedict, R. G. Botan. Rev. **19:** 229–320, 1953.

Nocardin

Produced by: Nocardia coeliaca (1).

Synonym: Possible relationship with trehalosamine.

Method of extraction: Broth-filtrate stirred with charcoal. Eluted with ether-95 per cent ethanol (1:1). Evaporated to dryness. Mycelium extracted with ether-ethanol (1:1). Production of the antibiotic (but not the mycelium) greatly stimulated by trehalose.

Chemical and physical properties: Crude substance. Water-soluble and thermostable.

Biological activity: Active on certain strains of mycobacteria, including *M. tuberculosis* var. *hominis* H37Rv. Active *in vivo* on mycobacteria in chick embryo, guinea pig, and mouse (1,2).

Toxicity: 25 mg of crude nocardin is more toxic to mice than 1.5 mg of streptomycin, subcutaneously (2).

References:

1. Emmart, E. W. Am. Rev. Tuberc. **56:** 316–333, 1947.
2. Emmart, E. W. *et al.* J. Bacteriol. **57:** 505–514, 1949.

Nocardorubin

Produced by: Nocardia narashinoensis (2).

Method of extraction: Extraction of the broth-filtrate with n-butanol at pH 2.0 to 3.0. Concentration of the solvent layer to syrup *in vacuo*. Syrup extracted with acetone; again concentrated to syrup. Extraction of the syrup with water; 10 per cent NaOH added dropwise until a deep red precipitate forms. Precipitate collected by centrifugation, washed with water, and dried. Nocardorubin can also be extracted with acetone from the mycelium.

Chemical and physical properties: Red at alkaline reaction, yellow at acid pH. Alkaline form is slightly soluble in dioxane, ethyl acetate, chloroform, and propylene glycol. It is almost insoluble in most organic solvents and water. The acid form is soluble in water and organic solvents. Carbohydrate and protein tests negative. Turns black at 180°C, but does not melt at 250°C. Aqueous solutions stable for at least 2 months at 27°C. Heating solutions at pH 2.0 reduces the activity in 5 to 15 minutes at 60°C. At pH values above 5.8, this treatment does not destroy the antibiotic activity.

Biological activity: Active against many gram-positive bacteria in a concentration of 0.001 to 5.0 μg per ml. Also active against mycobacteria and actinomycetes. Active against certain gram-negative bacteria at a concentration of 20 μg per ml. Activity against fungi low or *nil*. Limited activity against *D. pneumoniae* in mice. The development of resistance to nocardorubin is slow.

Toxicity: LD_0 (mice) about 154 mg per kg; LD_{100} about 230 mg per kg intraperitoneally.

References:

1. Aiso, K. *et al.* J. Antibiotics (Japan) **7A:** 1–6, 1954.
2. Endo, T. J. Antibiotics (Japan) **9A:** 228, 1956.

Noformicin

Produced by: Nocardia formica.

Synonym: Antibiotic MK 61.

Method of extraction: Adsorption from the culture-filtrate on activated carbon, and elution with 90 per cent aqueous methanol under acidic conditions. Concentration by chromatography over activated carbon or a cation exchange resin. The salt-free concentrate evaporated until crystals form (1).

Chemical and physical properties: Basic substance. Analysis of crystalline sulfate: $C_{17}H_{34}N_{10}O_5(SO_4)_2$; m.p. 265°C (decomposition) for the hydrochloride. Water-soluble. Dialyzable (1). Stable in solutions up to pH 8.0. Among the hydrolysis products, ammonia and glutamic acid have been identified. Nonreducing. No phenolic groups (1).

Biological activity: Prolongs life of mice infected with swine influenza, influenza A (PR 8), or influenza B (Lee). Treatment is effective whether the material is administered subcutaneously, intravenously, or orally. An effect is obtained when the material is administered as early as 24 hours before, or as late as 24 hours after infection. Best results obtained by treating the mice with multiple small doses for 3 days following infection. The agent is also active against mumps and against Newcastle disease virus (NDV) in embryonated eggs. No activity demonstrated against NDV infection in mice or against the nontransmissible pneumonia produced by NDV in mice. No activity against SK encephalomyelitis in mice, hog cholera in pigs, enteritis in mink, or hepatitis in dogs (1). Active on virus development in host cell, not on free virus. Inhibits the production of tobacco mosaic virus in leaf discs floated in solutions of the antibiotic. Active *in vivo* against southern bean mosaic virus and tobacco mosaic virus in intact plants (2, 3).

Toxicity: Mice tolerate daily doses of 5 mg per kg subcutaneously and intravenously; 12.5 mg per kg tolerated orally (1).

References:

1. Harris, D. A. *et al.* Antibiotics Ann. 1953–1954, p. 609–621.
2. Schlegel, D. E. and Rawlins, T. E. Phytopathology **44:** 328–329, 1954.
3. Gray, R. A. Phytopathology **45:** 281–285, 1955.

Novobiocin

Produced by: Streptomyces spheroides (2, 7), *S. niveus* (3, 11), *S. griseoflavus* (also produces an actinomycin (1, 30)), *S. griseus* (61), and *Streptomyces* sp. (22).

Synonyms: Cathomycin, streptonivicin, cardelmycin, antibiotic PA 93, crystallinic acid, vulcamycin (Italy), griseoflavin, albamycin, biotexin, inamycin, cathocin, antibiotic S 800.

Remarks: Novobiocin was reported to have been isolated in 1953 by Waga under the name griseoflavin. Waga's original description (1) leaves this open to some doubt, since unfortunately he published no ultraviolet or infrared spectrum, although Kuroya (3) clearly isolated novobiocin from Waga's culture 5 years later. The principal discrepancies between Waga's original description (apparently he isolated the sodium salt of novo-

biocin) and Na novobiocin are solubility in water and biological activity against *Diplococcus, Proteus, Mycobacterium,* and *B. subtilis.* By mutual agreement, the three groups who independently isolated this antibiotic under the names streptonivicin, cathomycin, and cardelmycin, have changed the generic name to novobiocin.

Method of extraction: I. Filtered broth evaporated to dryness. Residue dissolved in water and adjusted to pH 2.0. Precipitate which forms is dried, then triturated with acetone and filtered. Acetone solution evaporated to dryness *in vacuo* and residue triturated with methanol. Methanol filtered and evaporated *in vacuo.* Methanol-insoluble residue triturated with petroleum ether, then dissolved in dilute NaOH. Addition of HCl gives a precipitate. Dried precipitate extracted with ether, and ether evaporated off. Crystallized from aqueous acetone or ethanol (2). II. Whole broth adjusted to pH 8.0 and filtered. Filtrate extracted with amyl acetate at pH 6.0. Back-extracted into water at pH 10.0. Re-extracted into amyl acetate at pH 6.0. Crystallized from concentrated amyl acetate solution on addition of acetone and petroleum ether. Recrystallized from acetone, acetone-water, and acetone-hexane (30). III. Broth-filtrate extracted with methyl isobutyl ketone at pH 2.0. Extract washed with $NaHCO_3$, and extracted with ammonium hydroxide. Re-extracted from aqueous solution into ether at pH 2.0. IV. A super saturated solution of a crude salt of novobiocin in methanol or ethanol is obtained by concentration of the solvent solution, and a novobiocin salt-adduct with the solvent precipitates on cooling or seeding. Drying in the adduct in air gives purified sodium (or potassium) novobiocin.

Chemical and physical properties: Acidic substance. Pale yellow to white crystals existing in two polymorphic modifications: I. m.p. 152–156°C (decomposition) (most common); and II. m.p. 170–172°C (decomposition) (2), 172–174°C (22), or 174–178°C (decomposition) (3). Soluble in water at pH >7.5; insoluble at lower pH. Acidic form soluble in acetone, ethyl and amyl acetates, methanol, ethanol, and pyridine. Ultraviolet absorption spectrum maxima at 334 mμ (a = 40.7) in 0.01 N ethanolic (70 per cent H_2SO_4 (2); at 326 mμ ($E^{1\%}_{1cm}$ 356 to 367) and a shoulder at 290 mμ in 0.1 N HCl and methanol (90 per cent); at 238 mμ ($E^{1\%}_{1cm}$ 371 to 374) and 304 mμ ($E^{1\%}_{1cm}$ 334) with shoulders at 250 and 290 mμ in 1 per cent phosphate buffer pH 6.0; at 248 mμ (a = 36.2) and 308 mμ (a = 32.8) in 0.01 N ethanolic (70 per cent) phosphate buffer (pH 7.5); at 311 mμ (a = 53.2) in 0.01 N ethanolic (70 per cent) KOH; or at 307 mμ ($E^{1\%}_{1cm}$ 574 to 580) with a shoulder at 230 mμ in 0.1 N NaOH (2–4). Infrared spectrum given in references 12 and 22, and differs for the two forms. $[\alpha]_D^{25} = -27°$ (c = 1 per cent in NaOH) and $[\alpha]_D^{25} = -44°$ (c = 1 per cent in pyridine), both forms having the same optical activity (3). Mean refractive index, 1.595 ± 0.003 (4). $pK_a' = 4.3$ and 9.1 in water (12). Behavior in various systems on paper chromatography given in reference 11. Crystallographic data given in reference 12. Relatively stable; loses 23 per cent of its activity in saline solution after 2 weeks at 26°C (5). Does not form salts with ammonia and ordinary amines (12). $C_{31}H_{36}N_2O_{11}$: C = 60.62%; H = 5.91%; N = 4.54% (14). Structural formula of novobiocin (15, 16) given in Chapter 6. Hydrogenation yields "dihydronovobiocin," m.p. 163–165°C, with potentiometric titration and optical activity values similar to novobiocin. Ultraviolet absorption spectrum maximum at 328 mμ (in 0.01 N H_2SO_4 in 70 per cent ethanol) (3). Acid degradation under various conditions yields: (a) Cyclonovobiocic acid: pale yellow crystals; m.p. 288–290°C (decomposition); $pK_a' = 6.3$; optically inactive; $C_{22}H_{21}NO_6$ (3, 25, 33). (b) Novobiocic acid: m.p. 217–232°C; the aglycon of novobiocin (33). (c) The methyl-glycoside of 3-O-carbamylnoviose: m.p. 191–192°C; $C_{10}H_{19}NO_6$ (15, 25, 32); (d) The ethylglycoside of the same sugar: $C_{11}H_{21}NO_6$; m.p. 173–175°C (25). Originally noviose was termed novobiose, but this term was dropped, inasmuch as this implied a disaccharide (25). In dilute alkali, novobiocin isomerizes to isonovobiocin in an equilibrium reaction, as shown in Chapter 6. Since isonovobiocin is biologically inactive, the position of the carbamate group appears to govern the antibiotic activity of novobiocin (26). *Na novobiocin:* Glistening, snow-white platelets. Melts slowly above 220°C (decomposition) (12) or m.p. 210–215°C (decomposition) (25). Very soluble in water. Ultraviolet absorption spectrum like that of novobiocin. Infrared spectrum given in reference 12. *Ca novobiocin:* Nearly white, microcrystalline powder. Loses birefringence at >240°C, but does not melt up to 325°C (25). Less soluble in water than the sodium salt (28). *Sodium methanol adduct:* (See IV under "Methods of Extraction"). Needles; m.p. 225° ± 5°C (decomposition) with loss of birefringence at 10°C before melting. Less soluble in methanol than sodium novobiocin. *Sodium ethanol adduct:* Strongly birefringent; m.p. 217–222°C (decomposition).

Biological activity: Active on gram-positive bacteria and certain gram-negative bacteria such as *Hemophilus* and *Pasteurella.* Active at 25 to 50

μg per ml on *Proteus* and *Nocardia asteroides*, some atypical *Pseudomonas* and certain others, but otherwise inactive on gram-negative bacteria. Inactive on rickettsiae and viruses (9, 11). Slight antiprotozoal activity (8). Inhibits *Blastomyces dermatitidis* at 15 μg per ml (9), but not other fungi (8). Gives rise to long filaments in gram-negative but not gram-positive bacteria (18). Mg^{++} moderately reverses the activity of novobiocin against gram-negative but not gram-positive bacteria, as do Ca^{++}, Sr^{++}, and Ba^{++} to a lesser extent (23). Bound and inactivated by serum (59). Activity decreases with increasing pH (10). Synergistic activity with many antibiotics (7). Resistance develops rapidly (10). No cross-resistance with common antibiotics. Occasionally, increase of resistance to novobiocin is accompanied by a decreasing resistance to other antibiotics (5, 6). Dihydronovobiocin has activity *in vitro* and *in vivo*. Similar to novobiocin (25, 31). Active in mice on *Staph. aureus*, *Streptococcus hemolyticus*, *D. pneumoniae*, *Hemophilus*, *Pr. vulgaris*, and *Pasteurella multocida* (5, 6, 8). Conflicting reports on activity *in vivo* on *Sal. typhosa* and *K. pneumoniae* (6, 8). Some activity on downy mildew of cucumber (*Pseudoperonospora cubensis*) has been claimed (27).

Toxicity: Novobiocin: LD_{50} (mice) 262 to 300 mg per kg intraperitoneally (13), about 400 mg per kg (9) or 1 gm per kg orally (13), and 407 to 424 mg per kg intravenously. LD_{50} (guinea pigs) 11.5 mg per kg intraperitoneally, and 27.8 mg per kg subcutaneously. Moderately toxic to rabbit skeletal muscle, dog liver and kidney at high doses; local tissue injury on repeated subcutaneous injections (13, 22). Side effects in human beings include rash, leukopenia, and eosinophilia (20). Highest concentration permitting migration of epithelial cells in tissue culture is 200 μg per ml (34). *Dihydronovobiocin:* LD_{50} (mice) 325 mg per kg intravenously, and about 1100 mg per kg orally (22).

Utilization: Effective in certain infections caused by some gram-positive organisms, but primarily useful in infections, especially staphylococcal, caused by organisms resistant to other antibiotics. Resistance to novobiocin develops rapidly in clinical use. Recommended for use concomitantly with other antibiotics (19). Not effective in infections caused by enterococci or actinomycetes (20). Possible use in certain *Proteus* infections (17) and brucellosis (29).

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Novomycin

Produced by: Streptomyces roseochromogenes.

Method of extraction: Extracted with the use of a cation exchange resin.

Chemical and physical properties: Basic substance. *Reineckate:* m.p. 187–188°C. Water-soluble. Positive ninhydrin test. Negative maltol, Molisch, Fehling, $FeCl_3$, and biuret tests. Optically inactive. Ultraviolet spectrum shows only end-absorption. Differentiated from fradiomycin, neamine, and roseomycin by paper chromatography.

Biological activity: Active on gram-positive and gram-negative bacteria (0.75 to 1.5 μg per ml) and mycobacteria (1.25 to 2.5 μg per ml.).

Toxicity: LD_{50} (mice) 667 to 1334 mg per kg intravenously.

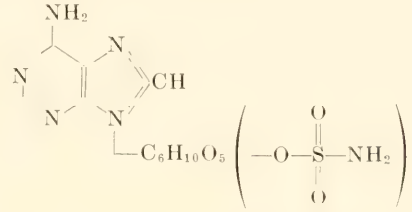
Reference: 1. Maeda, K. *et al.* Japan. J. Bacteriol. **9:** 600, 1954.

Nucleocidin

Produced by: Streptomyces calvus (2, 5).

Method of extraction: Adsorbed from broth on a Darco G-60-Celite 545 mixture at pH 7.0. Eluted with 95 per cent aqueous acetone, acidic or basic alcohols, aqueous acidic alcohols, "Cellosolves," or dilute acids. Eluate concentrated *in vacuo* to a small volume, then (a) lyophilized; or (b) taken up in 50 per cent aqueous acetone and chromatographed on activated charcoal or charcoal-Celite; or (c) washed with butanol at pH 2.0, adjusted to pH 7.0, saturated with ammonium sulfate, and extracted with butanol. Butanol concentrated to remove the solvent, and residue lyophilized. Chromatographed from butanol-saturated water mixed with diatomaceous earth at pH 2.0 on Celite and developed with almost water-saturated butanol. Crystallized from dilute aqueous solution at pH 4.0 by cooling to 4–5°C and seeding. Can also be extracted from an ammonium sulfate-saturated broth with n-butanol or acetone (1, 5).

Chemical and physical properties: Amphoteric (1) or weakly basic (4) substance. White, thread-like crystals. Soluble in methanol, acetone, and water at pH 9.2 and 3.2. Less soluble in water at pH 6.5. Slightly soluble in n-butanol, ethyl acetate and benzene; practically insoluble in ether. $\lambda_{max}^{0.1\ N\ NaOH}$ 259 mμ ($E_{1cm}^{1\%}$ 406); $\lambda_{max}^{0.1\ N\ HCl}$ 255 to 257 mμ ($E_{1cm}^{1\%}$ 392); $\lambda_{max}^{water\ (pH\ 7)}$ 256 mμ (ϵ = 15,500). Infrared spectrum given in references 1 and 5. $[\alpha]_D^{24.5} = -33.3°$ (c = 1.05 per cent in a 1:1 ethanol-0.1 *N* HCl mixture). Stable at pH 3, 7, and 9 at room temperature for 24 hours. Destroyed by boiling. $C_{11}H_{15\text{-}16}N_6O_8S$: C = 34.03%; H = 4.05%; N = 21.60%; S = 8.30%; OCH_3 = 0%; N—CH_3 = 0%; (1, 5). Hydrolysis with ethanolic HCl yields adenine. Nucleocidin may be the glycoside of adenine in which sulfamic acid is bound to the carbohydrate moiety as an ester. Partial structure (4):



Picrate: Bright yellow crystals; m.p. 143–144°C (uncorrected, slight decomposition). λ_{max}^{EtOH} 253 and 356 mμ. Forms a white hydrochloride, an amorphous sulfate, and a colored crystalline helianthate (5).

Biological activity: Active *in vitro* on gram-positive and gram-negative bacteria, including mycobacteria, *Pseudomonas*, and *Erwinia*. Active on *Endamoeba histolytica*. Active *in vivo* on *Trypanosoma equiperdum* infections in mice and rats, but not on *Streptococcus pyogenes* infections (1, 3, 5).

Toxicity: Very toxic. LD_{50} (mice) 0.2 mg per kg intraperitoneally (1).

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5. British Patent 815,381, June, 24, 1959.

Nybomycin

Produced by: Streptomyces sp. (1, 2).

Method of extraction: I. Washed mycelium extracted with boiling 95 per cent ethanol; the extracts dried *in vacuo*. Impurities removed with

boiling Skellysolve C, cold ether, and cold acetone; residue extracted with boiling ethanol, from which crude nybomycin crystals separate on cooling. Filtered broth successively extracted with n-butanol at pH 6.0, or, after acidification to pH 2.5 and filtration, with chloroform. After evaporation to dryness of this extract, crude nybomycin is obtained in the same way as from mycelium. Pure nybomycin precipitates from a concentrated HCl solution which has been treated with charcoal, after dilution with 5 to 10 parts water. Recrystallization from boiling acetic acid solution by cooling, or on addition of water, acetone, or other organic solvents (1). II. Filtered mycelium extracted with boiling dimethylformamide, boiling n-butanol, or water at 120–126°C under 20 to 25 pounds of pressure. Crystalline crude nybomycin precipitates from these extracts on cooling, and is washed with a hot aqueous solution of sodium versenate (to remove calcium salt) and hot water; recrystallized from dimethylformamide (2).

Chemical and physical properties: Weakly basic substance. Exists in two interconvertible forms: I. white needles, rods, or rhomboid crystals (crystallized from glacial acetic acid); II. crystallized from dimethyl formamide or concentrated HCl-water; characterized by their different infrared spectra in potassium bromide briquette. Sublimes *in vacuo* at 250°C with no change of ultraviolet absorption spectrum or biological activity. Darkens at about 330°C without melting. Soluble in concentrated HCl and sulfuric acids, and somewhat soluble in dichloroacetic acid. Recovered from these solvents unchanged on addition of water. Very slightly soluble in boiling dimethylformamide, boiling water, pyridine, and methyl isobutyl ketone. Insoluble (at 24°C or boiling point) in 2-propanol, acetonitrile, benzene, chloroform, n-butyl ether, dioxane, ethyl acetate, lower alcohols, water, 1 *N* NaOH, and 1 *N* HCl (1, 2). Ultraviolet absorption spectrum maxima at 266 and 285 mμ (ethanol) (1) or at 222, 266, 286, and 369 mμ, with inflections at 236, 298, and 355 mμ (a = 61.8, 103.1, 136.2, 41.1, 50.2, 107.7, and 37.4, respectively) in dimethylformamide, methanol, 0.01 *N* ethanolic H_2SO_4, or 0.01 *N* ethanolic KOH (2). Infrared spectra of Forms I and II given in reference 2. Optically inactive. Negative $FeCl_3$, biuret, ninhydrin, Tollen, Benedict, neutral permanganate, Ehrlich (*p*-dimethylaminobenzaldehyde), Ehrlich, Pauly, and Br_2 in CCl_4 tests. $C_8H_7O_2N$ or $C_{16}H_{14}N_2O_4$: C = 64.41%; H = 4.87%; N = 9.12%. Molecular weight, 164 (1) or 298 (2). *Hydrochloride:* Yellow substance *Acetate:* White. crystals; m.p. 235–237°C. Ultraviolet absorption spectrum same as the base. *Dichloroacetate:* Crystalline; m.p. 253–256°C (1, 2). On paper chromatography, nybomycin gives a blue fluorescence in neutral and alkaline systems and a yellow fluorescence in acid (2).

Biological activity: Nybomycin and its salts are active on gram-positive bacteria, certain gram-negative bacteria, certain fungi, and phages. Against bacteria, nybomycin and its acetate differ in activity, the base being active against certain gram-positive organisms at 1.5 to 32 μg per ml and the acetate being active against the same organisms at 0.8 to 8 μg per ml. The acetate is also active at 4 to 15 μg per ml against a number of bacteria resistant to nybomycin. Both are active against *K. pneumoniae* (2 μg per ml), but not against other gram-negative bacteria. Nybomycin is active against *M. tuberculosis* H37Rv at 0.5 μg per ml. Nybomycin acetate, but not nybomycin, is active on *A. niger* (6.2 μg per ml) and *Endothia parasitica* (12.5 μg per ml). Both are active on *Microsporum canis* (10 μg per ml), but not on other fungi tested. Both are active on a variety of phages, including certain staphylophages, coliphages, *B. cereus* phage, *Staph. aureus* phage, and *S. griseus* phage. No obvious correlation between antiphage and antibacterial (antihost) activity. Certain coliphages are stimulated. Neither nybomycin nor its salts are active in mice on *Streptococcus hemolyticus*, *Pasteurella multocida*, *Sal. typhi*, *Sal. paratyphi*, or *M. tuberculosis*. Nybomycin, but not its acetate, gives partial protection against *K. pneumoniae* (mice). Also inactive on Newcastle disease virus NJ-KD, vaccinia (eggs), influenza A (PR 8, mice and eggs), and SK poliovirus (mice) (1, 3).

Toxicity: Nybomycin: LD_{50} (mice) 650 mg per kg intraperitoneally. *Nybomycin acetate:* LD_{50} (mice) 200 mg per kg intraperitoneally (3).

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Nystatin

Produced by: Streptomyces noursei (5, 30); this organism also produces cycloheximide (1). *Streptomyces* sp. (40). *S. albulus*; this strain also produces two forms of cycloheximide and antitumor antibiotic E 73 (37).

Synonyms: Fungicidin (1). Closely related to antimycoin.

Method of extraction: I. Mycelium heated at

70°C for 10 minutes, then extracted with methanol, butanol, ethanol, n- or isopropanol, or methanol-ethanol (1:1) (5). Fractional precipitation with ethyl acetate. Fractional precipitation from methanol with ether (1). II. Methanolic or acetone extracts of mycelium concentrated *in vacuo*. Residue suspended in 0.85 per cent NaCl-butanol (1:1). Precipitate at interface washed with saline, dissolved in warm methanol, cooled, and reprecipitated with saline. Precipitated from methanol with ethyl acetate (30). III. Purified by treatment with hot ethyl acetate, then extracted with warm 70 per cent aqueous acetone. Slow cooling gives an amorphous precipitate, followed by active needles. Distribution of material in a biphasic system (butanol-methanol-water-hexane, 50:50:-35:15) and air evaporation yield crystals at the interface with little inactivation (2). IV. Forms a complex with $CaCl_2$ in methanol, giving separation from impurities. Addition of water to this $CaCl_2$-methanol solution precipitates crystalline nystatin (25, 34). V. Broth and mycelium extracted with n-butanol. Extract distilled off *in vacuo* at <45°C. Residue extracted with methanol as in Step I (30). VI. Isopropanol added to whole broth; pH adjusted to 5 with H_3PO_4; stirred; pH adjusted to 7 with NaOH; and mixture filtered. Isopropanol evaporated off *in vacuo* at <30°C to precipitate nystatin (29). Precipitate slurried in 20 per cent aqueous isopropanol containing 0.5 per cent sodium hexametapyrophosphate (weight per volume) at 60°C, and cooled to 10°C with mild agitation to precipitate nystatin (34).

Chemical and physical properties: Amphoteric conjugated tetraene. Pale yellow microcrystals (9) or needles (26). Gradual decomposition above 160°C. No melting up to 250°C (2). Soluble in pyridine, propylene glycol, dimethylformamide, and dimethylacetamide (2, 9). Soluble with inactivation in glacial acetic acid, 0.05 *N* methanolic HCl, or NaOH. Solubility in polar solvents increased in presence of 10 to 20 per cent water. Slightly soluble in methanol, ethanol, butanol, and dioxane. Insoluble in water, ether, chloroform, hexane, and acetone (2, 12). Ultraviolet absorption spectrum maxima (methanol) at 235, 291, 304, and 319 mμ (E = 298, 536, 754, and 596, respectively) (30). Infrared spectrum given in reference 30. $[\alpha]_D^{25}$ = +21° (pyridine) or −10° (glacial acetic acid) (2). Positive Benedict, Molisch, and carbazole tests. $KMnO_4$, iodine-KI, and Br_2-CCl_4 decolorized. Slowly positive Schiff test. Negative $FeCl_3$, biuret, ninhydrin, Millon, phenol, Fehling, Tollen, and 2,4-dinitrophenylhydrazine tests. Blue color with $FeCl_3$-$K_3Fe(CN)_6$. In concentrated H_2SO_4, color changes from violet to blue to black (2, 12, 30). Pink in phosphoric acid; yellow in hydrochloric acid (30). Withstands 55°C for 1 hour at pH 5 to 8 in a methanolic solution. Inactivated by heat, light, oxygen, and excessively high or low pH (2, 30). Neutral equivalent, 956 (glacial acetic-perchloric acids) (2). Potassium and sodium salts insoluble in water and unstable; hydrochloride is water-soluble and stable (2). Nitrogen-containing moiety, which is also present in amphotericin B, is mycosamine (27). *Nystatin* $C_{46}H_{77}NO_{19}$: C = 58.5%; H = 8.23%; N = 1.64%; C—CH_3 = 5.86%. Contains no methoxyl, acetyl, or N—CH_3 groups. Contains the same chromophore as methyl-α-parinarate. Contains a diene system in addition to its tetraenic chromophore, carboxyl, and lactone functions (12, 25).

Biological activity: In vitro: Active on fungi and yeasts (1, 30), including a variety of plant pathogens (33). Inactive on bacteria (1). Molluscacide (10). Active on *Endamoeba histolytica* (9). Activity reversed by cysteine (2). Development of resistance is negligible. Cross-resistance with other polyenic antibiotics (15). *In vivo:* Active on moniliasis in mice, rabbits, and chickens (3, 13, 24) and on histoplasmosis, coccidioidomycosis, sporotrichosis, cryptococcosis, blastomycosis, and *Mucor pusillus* infections, all in mice (4, 7, 8, 32). *In plants:* Protects beans from anthracnose (*Colletotrichum lindemuthianum*) and partially protects them from rust (*Uromyces phaseoli* var. *typica*). Reduces post-harvest decay of strawberries (*Botrytis* and *Rhizopus*) (22) and peaches (19). Active on downy mildew of cucumber (*Pseudoperonospora cubensis*) (21) and broccoli (*Peronospora parasitica*) (23). Active on peach brown rot (*Sclerotinia fructicola*) (20). Active on *Botrytis* infection of orchids (*Cattleya*) (38), roses (35), and tulips (36). Some control of pink snow mold (*Fusarium nivali*) (31).

Toxicity: Crude nystatin: LD_{50} (mice) 20 to 26 mg per kg intraperitoneally. Subcutaneously, 2 gm per kg is tolerated, but with necrosis and induration at site of inoculation (1). Ten μg per ml is nontoxic to HeLa, monkey kidney, and normal human epithelial cells (11). *Purified nystatin:* LD_{50} (mice) 8 to 14 mg per kg intraperitoneally; (rats) 24.2 to 24.6 mg per kg. Mice tolerate 2.3 to 3.5 gm per kg orally (9).

Utilization: Prophylaxis against yeast overgrowth during administration of antibacterial antibiotics (6). Moniliasis. Intestinal amebiasis (9). Coccidioidomycosis (16). Trichophytosis and epidermophytosis (14). Does not give rise to resistant *Candida* strains during treatment (18). Some effect in preventing moniliasis in poultry (39).

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Oleandomycin

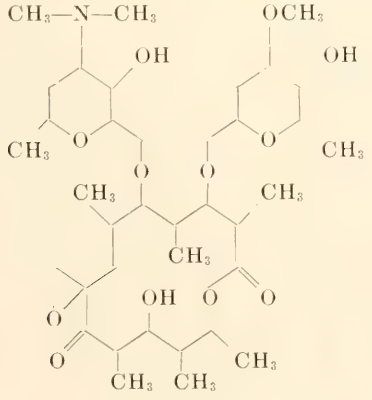
Produced by: Streptomyces antibioticus (4). This organism produces a minor component in the culture-broths, identified as oleandomycin chlorohydrin: colorless, felt-like crystals; m.p. 150–153°C (decomposition) (hydrochloride) (16, 18).

Synonyms: Antibiotic PA 105, matromycin, romicil, amimycin, antibiotic RO 2-7638.

Method of extraction: I. Mycelium extracted with methyl isobutyl ketone. Extract concentrated *in vacuo*. Concentrate extracted into water (pH 2.0). Aqueous phase washed with benzene to remove the methyl isobutyl ketone, then extracted with ether at pH 6.5. Removal of ether by distillation gives crystals. Recrystallized from hot ethyl acetate (4). Base crystallized from aqueous methanol or acetone (18). II. Filtered broth extracted with methyl isobutyl ketone. Back-extraction with dilute aqueous acid. Extraction with methyl ethyl ketone at pH 9.0 Hydrated oleandomycin hydrochloride precipitated upon the addition of HCl (1).

Chemical and physical properties: Macrolide (12). Basic substance. *Base:* Colorless prisms, m.p.

110°C (decomposition). Very soluble in methanol, ethanol, acetone, and butanol. Soluble in dilute aqueous acids and moderately soluble in water and ligroin. Insoluble in hexane, carbon tetrachloride, and n-dibutyl ether. More soluble in aqueous alkaline solution than erythromycin or carbomycin. Ultraviolet absorption spectrum maximum at 290 to 295 mμ ($\epsilon = 50$), a broad, low intensity peak (4, 16, 18). Infrared spectrum given in references 1 and 4. $[\alpha]_D = -65° \pm 1°$ (c = 2 per cent in methanol). Positive Fehling (slow) and Tollen tests. Negative $FeCl_3$ and ninhydrin tests. Rf = 0.5 on paper chromatography with benzene saturated with formamide. $pK_a' = 8.5$. $C_{35}H_{61-63}O_{12}N$ (12, 16): C = 61.11%; H = 8.98%; N = 2.15%. Molecular weight, about 715. Equivalent weight, 690 (1, 16, 18). Forms a crystalline solvate with chloroform, m.p. 120–122°C (decomposition) in which the chloroform is tenaciously bound, as well as other solvate-hydrate complexes. Methanolysis of oleandomycin under mild acidic conditions gives "desoleandomycin," $C_{28}H_{49}NO_9$, a colorless substance, m.p. 116–118°C, and $C_8H_{16}O_4$, the methylglycoside of L-oleandrose (2-desoxy-L-glucomethylose-3-methyl ether). Acid hydrolysis products include desosamine (18). *Oleandomycin hydrochloride* (anhydrous): m.p. 125–128°C (1) or 134–135°C (4). *Hydrochloride* (Dihydrate): Crystalline; m.p. 134–135°C. Very soluble in water. $[\alpha]_D = 56.8°$ (c = 2 per cent in methanol). Stable in aqueous solution at room temperature for several hours, over a wide range of pH. Stable in the dry state or in anhydrous solvents. Unstable to acid pH on heating (4, 18). *Sulfate:* Long, white needles. Soluble in water (4). *Phosphate:* Crystalline; m.p. 150°C (decomposition). Water-soluble (16). Oleandmycin is composed of a polyhydroxy, polymethyl ketolactone ("oleandride") glycosidically bound with the desoxy sugars L-oleandrose and desosamine (3). Partial structure is given in reference 16. Complete structure (21):



Biological activity: In vitro: Active on gram-positive bacteria at <0.19 to 6.25 μg per ml. Inactive on gram-negative bacteria, except for the following: *Listeria monocytogenes* (3.12 μg per ml), *Neisseria* (3.12 to 6.25 μg per ml), *Br. bronchiseptica* (7 to 25 μg per ml), and *H. influenzae* (0.078 μg per ml) (1,4). Not active on *C. albicans*. Activity diminished by serum and urine (7). Synergistic action with tetracycline on certain gram-positive organisms *in vitro* and *in vivo* (6). Cross-resistance with erythromycin, spiramycin, carbomycin, and streptogramin (5). *In vivo:* Active on *Leptospira icterohaemorrhagiae* infections (hamsters) (14); *Streptococcus hemolyticus, D. pneumoniae, Staph. aureus, Enterococcus, Sal. typhimurium* (mice), *Rickettsia rickettsii* (eggs) and other rickettsiae, as well as psittacosis (eggs, mice) and other intracellular parasites (1, 4, 9). Active on certain protozoa (1). Active, at high doses, on tuberculosis and syphilis (7, 8). Not active on trypanosomiasis, trichophytosis (8), *Rickettsia akari*, SK encephalomyelitis, or influenza A viruses (9). Stimulates growth of chicks (15) and turkey poults (17).

Toxicity: Hydrochloride: LD_{50} (mice) 460 to 600 mg per kg intravenously, about 4 gm per kg by gavage, 8.2 gm per kg orally, and 2.5 gm per kg subcutaneously (8, 13). LD_{50} (rat) 376 ± 5 mg per kg (12) or 550 mg per kg (1) intravenously, 10.0 gm per kg subcutaneously, and >10.0 gm per kg orally (8). Toxicity to hamsters increased over a a period of 1½ years from the time the drug was introduced into the laboratory (14). Highest concentration permitting migration of epithelial cells in tissue culture is 20 mg per ml (20).

Utilization: Used in infections caused by gram-positive organisms and antibiotic-resistant staphylococci (2, 9–11). Used in combination with other antibiotics (6).

Triacetyloleandomycin

Produced by: Acylation of oleandomycin.

Chemical and physical properties: $C_{41}H_{67}NO_3$. Insoluble in distilled water and buffered aqueous solutions (pH 4 to 7). Ultraviolet spectrum like that of oleandomycin. Can be separated from parent compound by paper chromatography (formamide-saturated benzene) (16).

Biological activity: Has about one third the activity of oleandomycin on *Sarcina lutea*. Gives higher blood levels in human beings than oleandomycin (16).

Toxicity: Well tolerated by human beings (19).

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Oligomycins

Produced by: Streptomyces sp. resembling *S. diastatochromogenes* (1), and *Streptomyces* sp. (7).

Synonym: The originally described oligomycin contained mostly oligomycin A (10).

Remarks: All the antibiotic activity could be retained in the mycelium if the pH of the culture were controlled (7).

Method of extraction: I. Broth clarified with Celite 535 and adjusted to pH 2.0 with HCl. Precipitate which forms extracted with ethyl acetate. Extract decolorized with Darco G-60, and distilled off *in vacuo*. Residue treated with Skellysolve A and ground to a brown powder. Crystallized from glacial acetic acid-cold distilled water, then from ethanol-water. Purification and separation of the components by chromatography on Celite 545 pretreated with the aqueous phase of Skellysolve B - dioxane - ethanol - water mixture (200:72:26:18.5) and developed with the organic phase, giving components C, A, and B, in that order (11). *Component A-containing fractions:* Evaporated and cooled to give a semisolid product. To an ether solution of this, Skellysolve B is added to incipient precipitation. Crystallized on standing to give Form I of A (AI); m.p. 139–141°C. Slow crystallization from ether-Skellysolve B mixtures or dilute acetic acid solutions gives Form II (AII); m.p. 150–151°C (decomposition). Recrystallization of AII from acetone-methanol gives AI. *Component B:* Recrystallized from methanol to give Form I (BI); m.p. 160–161°C. BI changed to BII, m.p. 169–170°C, on standing at room temperature for several months. Recrystallization of BII from methanol gives BI. *Component C:* Crystallized from ether-petroleum ether (1:1) (7).

Chemical and physical properties: Complex containing three components, A, B, and C. Neutral unsaturated alcohols possibly containing ketone groups. *Complex:* Colorless hexagonal crystals; m.p. 135.8–136.3°C. Soluble in most common organic solvents but not in petroleum ether, cyclohexane, or water. $[\alpha]_D^{25.8} = -51°$ (c = 3 per cent in methanol) (1). *Oligomycin A:* Exists in two crystalline forms, AI and AII (see "Method of Extraction"). AI: Long needles; m.p. 140–141°C. AII: hexagonal crystals; m.p. 150–151°C (decomposition). Both very soluble in acetone, soluble in ethanol, ether, and glacial acetic acid; scarcely soluble in benzene; insoluble in water and Skellysolve B. $[\alpha]_D^{23} = 54.5°$ (c = 4.4 per cent in dioxane) (AII). AI: C = 67.76%; H = 9.51%; O = 22.30%; C—CH_3 = 16.4%; $C_{24}H_{40}O_6$. Diacetate: m.p. 112–113°C. $[\alpha]_D^{24} = -86.1°$ (c = 1.74 per cent in ethanol). *Oligomycin B:* Two crystalline forms, BI and BII (see "Method of Extraction"). BI: Platelets; m.p. 160–161°C. $[\alpha]_D^{23.5} = 49.5°$ (c = 1.03 per cent in methanol). C = 66.60%; H = 9.07%; O = 2.40%; C—CH_3 = 18.9%. $C_{22}H_{36}O_6$. BII: m.p. 169–170°C. Both very soluble in acetone; soluble in benzene and ether; scarcely soluble in ethanol and glacial acetic acid; insoluble in water and Skellysolve B. Diacetate: m.p. 135.5–136.5°C. $[\alpha]_D^{25} = -66.6°$ (c = 0.63 per cent in dioxane). *Oligomycin C:* Rod-shaped crystals; m.p. 198–200°C. Very soluble in acetone and glacial acetic acid; soluble in ethanol and ether; scarcely soluble in benzene; insoluble in water and Skellysolve B. $[\alpha]_D^{23} = -80.7°$ (c = 3.70 per cent in dioxane). C = 69.9%; C—CH_3 = 19.3%. $C_{28}H_{46}O_6$. *A, B, and C:* All have

the same ultraviolet absorption spectrum, with maxima at 225 and 232 ± 0.5 mμ (in absolute ethanol). Molar extinction values; A, 20,200 and 18,200; B, 18,800 and 17,000; and C, 23,200 and 21,600, respectively. Inflections at 220, 240 ± 0.5, and 285 mμ (A and C) or 295 mμ (B). Infrared spectrum and crystallographic data given in reference 7. All give a yellow to cherry-red color with concentrated H_2SO_4, the color varying with the concentration. Positive 2,4-dinitrophenylhydrazine, Fehling, ammoniacal silver nitrate, bromine, and $KMnO_4$ tests. Negative tests for phenol, steroid, coumarin, flavone and flavone-related compounds (1, 7). Thermostability at 95°C: C > A > B (6).

Biological activity: Active on certain fungi, including *Tilletia caries* (2, 10), but not on bacteria (1, 7). In general the activity of the components is quantitatively A > B > C, and qualitatively the same, although A is active on *Rhodotorula glutinis* and B and C are not. The *diacetate* is inactive on *Glomerella cingulata* (only organism tested) (7). Active on the soil nematode *Rhabditis briggsae* (1). Effective on post-harvest decay (*Botrytis* and *Rhizopus*) of strawberries (9), downy mildew (*Phytophthora phaseoli*) and stem anthracnose (*Colletotrichum truncatum*) of lima beans, rust (*Uromyces phaseoli* var. *typica*) and anthracnose (*C. lindemuthianium*) of beans (4), tomato early blight (*Alternaria solani*) (3), and brown rot (*Sclerotinia fructicola*) of peach (5). Blocks a reaction involved in phosphate transfer, but not electron transport *per se*, inhibiting respiration and phosphate uptake with certain substrates and inhibiting mitochondrial adenosine triphosphate (8).

Toxicity: LD_{50} (mice) 1.5 mg per kg (A), 2.9 mg per kg (B), and 8.3 mg per kg (C) intraperitoneally (7).

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Oxytetracycline

Produced by: Streptomyces rimosus strains (15, 27, 69); *S. platensis*, closely related to *S. hygroscopicus* (56, 84); *S. armillatus* (65) (this may be the same culture as that described in reference 83 as *Streptomyces* sp.); *S. vendargensis* (86) (this culture produces vengicide, and five or six other antibiotics); *S. gilvus: Streptomyces* sp. resembling *S. griseolus*; and *Streptomyces* sp. One of these cultures produces a heptaene and a basic antibiotic active on gram-positive bacteria (66, 85).

Synonym: Terramycin.

Method of extraction: IA. Whole broth adjusted to pH 2.0 and filtered. Filtrate adjusted to: (a) pH 3.5 and extracted with phenyl Cellosolve; or (b) pH 9.0 and extracted with n-butanol, benzyl alcohol, amyl alcohol, or phenyl Cellosolve. Extract concentrated *in vacuo* at 50°C and extracted with 0.1 *N* HCl. Extract neutralized to precipitate oxytetracycline. Purified by chromatography on Florisil from a dilute HCl solution, and developed with water, followed by acetone. Acetone fractions diluted with water, acidified to pH 2.0, concentrated under reduced pressure to remove the solvent, then freeze dried. Powder dissolved in water, adjusted to pH 7.5, and extracted with n-butanol. Re-extracted into 0.05 *N* HCl. Extract concentrated *in vacuo* to precipitation (2, 15, 83). IB. After acidification and filtration of the whole broth, a sequestering agent, such as citric acid, sodium tetraphosphate, or ethylenediaminetetraacetic acid, is added to the filtrate to bind the multivalent metallic ions with which oxytetracycline forms complexes (see II). The whole adjusted to pH 10.1 and extracted with butanol. Butanol adjusted to 5.3 to precipitate oxytetracycline (54). IIA. Precipitated as the mixed barium-magnesium salt from culture-filtrate at pH 8.5. Precipitate suspended in water, and salt complex cleaved by acidification to pH 1.5 with sulfuric acid. Adjusted to pH 3.0, seeded, then adjusted successively to pH 5.0 and 7.0 to precipitate oxytetracycline. Purified by salt conversion or as in IV (18). IIB: Precipitated from culture-filtrate at pH 9.0 in the presence of multivalent metallic ions (as a metallo-organic salt) with organic amine bases such as octylamine, or quaternary ammonium halide salts such as alkyl and alkenyl trimethylammonium chlorides with eight

to eighteen carbons, such as contained in the various "Arquads" (see chlortetracycline). The complex is cleaved by dissolving in methanol at acidic pH, and filtering (58). III. Adsorbed from broth-filtrate on activated carbon at a pH near neutrality. Eluted with butanol-saturated water at pH 1.5. Eluate adjusted to pH 6 to 9, and (a) freeze dried; or (b) extracted into butanol at pH 9, extract concentrated, and extracted with aqueous acid. Aqueous extract adjusted to pH 6 to 9 with a base or Amberlite IR4 (15). IV. Can be precipitated as the salt of an organic sulfonic acid from broth-filtrates; as the picrate from acidic aqueous solution; as the orange II salt or other arylazosulfonic acid salt and recovered by treatment with $BaCl_2$ (15).

Purification: I. Solution in dilute aqueous HCl (pH 2.5) filtered, and treated with NaCl and shaking. Solid which separates dissolved in methanol and water added. Cooled to give crystals. II. Countercurrent distribution (butanol-water adjusted to pH 3.0 with HCl; 1:1) (15). III. Acid solutions (from IIA) purified by addition of potassium ferrocyanide to precipitate contaminating inorganic and organic substances (57).

Chemical and physical properties: Amphoteric substance (1). *Andhydrous Base:* Pale yellow substance; m.p. 184.5–185.5°C (decomposition) (1, 18). *Dihydrate:* Thick hexagonal plates or thick needles; m.p. 181–182°C. Soluble in methanol, ethanol, acetone, propylene glycol, and dioxane (1, 14). Solubility in organic solvents decreases on addition of water; little increase in solubility on heating (2). Sparingly soluble in water (0.25 mg per ml at 25°C), butanol, 90 per cent aqueous acetone, and 95 per cent methanol (14). Insoluble in ether and petroleum ether (1). Ultraviolet absorption spectrum maxima in: *methanol:* 270 ($E^{1\%}_{1cm}$ 361) and 370 mμ ($E^{1\%}_{1cm}$ 343); *methanolic 0.01 N HCl:* 270 ($E^{1\%}_{1cm}$ 353) and 359 mμ ($E^{1\%}_{1cm}$ 267); *methanolic 0.01 N NaOH:* 245 ($E^{1\%}_{1cm}$ 415), 264 ($E^{1\%}_{1cm}$ 405), and 375 mμ ($E^{1\%}_{1cm}$ 352); *dilute H_3PO_4(pH 1.7):* 268 ($E^{1\%}_{1cm}$ 379) and 353 mμ ($E^{1\%}_{1cm}$ 277); *0.1 M phosphate buffer (pH 4.5):* 249 ($E^{1\%}_{1cm}$ 240), 276 ($E^{1\%}_{1cm}$ 322), and 353 mμ ($E^{1\%}_{1cm}$ 301). Fluoresces bright yellow in ultraviolet light (2, 15, 18). Infrared spectrum given in references 2, 15, and 18. $[\alpha]^{25}_{D} = -196.6°$ (c = 1 per cent in 0.1 *N* HCl); −2.1° (c = 1 per cent in 0.1 *N* NaOH); +26.5° (c = 1 per cent in methanol) decreasing to +11.3° after 16 hours; 0° (c = 1 per cent in methanol containing 1 per cent boric acid); −234.2° (c = 0.5 per cent in methanol containing 1 per cent saturated $CaCl_2$-methanol solution) (2, 18). Positive $FeCl_3$, Pauly, Friedel-Crafts, Fehling, Molisch, modified Fearon-Mitchell, and amino antipyridine (phenols) tests (2, 24). Gives a deep red color with H_2SO_4 and $NaNO_2$ and a similar color with diazotized β-naphthylamine (24). Negative tests with carbonyl reagents and negative furfural (24). Most stable at pH 2.5; less stable at neutrality and alkaline pH (2). $pK_a' = 3.49$, 7.55, and 9.24 (18). Crystallographic data given in reference 15. X-ray measurements of various salts given in reference 37. $C_{22}H_{24}N_2O_9$. Molecular weight, 440 ± 30 (18). C = 53.05%; H = 5.91%; N = 5.64% (1). Structural formula (40) given in Chapter 6. 4-Dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene carboxamide. Alkaline degradation products include ammonia and dimethylamine. Carried out in the presence of zinc, alkaline hydrolysis yields, among other products: tribasic *terracinoic acid* (4-carboxy-5-hydroxy-3-methyl indanone-2-acetic acid), $C_{13}H_{12}O_6$, white crystals, m.p. 232–234°C (decomposition); a phenolic lactone, 7-hydroxy-3-methylpthalide, m.p. 110–112°C; acetic acid; and carbon dioxide (17, 19). Mild acid hydrolysis yields a yellow crystalline, optically active rearrangement product, *α-apoterramycin*, which is a stronger acid than oxytetracycline ($C_{22}H_{22}N_2O_8$); *HCl:* m.p. 198–202°C (decomposition); and its stereoisomer, *β-apoterramycin* (17, 39). Treatment with strong acid immediately gives an insoluble red product (24). Strong acid hydrolysis yields dimethylamine, CO_2, and "terrinolide," $C_{20}H_{15}NO_8$; m.p. 210–213°C (38). Other information on degradation products given in references 24 and 54. *Hydrochloride:* Bitter-tasting, bright yellow needles (from methanol) or platelets (from water) (2, 18); m.p. 190–194°C (93). Soluble in acetone, methanol, ethanol, and other polar organic solvents (2). Soluble in water, but excess acid must be added to pH 1.5 or the free base separates on standing (18). Least soluble in water at pH 5.0 (93). Insoluble in ether and petroleum ether (14). *Disodium and dipotassium salts:* Lemon-yellow crystals. Aqueous solutions are brown, becoming darker on standing. Soluble in water and insoluble in ethanol (4, 18, 24). *Calcium and magnesium salts:* Slightly soluble in water (18). Oxytetracycline also forms mixed salts (*e.g.*, barium-calcium), complexes with such salts as $CaCl_2$, a triacetyl derivative, and a crystalline pentabromo derivative (18, 24).

Biological activity: In vitro: Active on gram-positive and gram-negative bacteria, including clostridia, mycobacteria, and *Actinomyces*, and certain protozoa, rickettsiae, and psittacosis. Not active on *Proteus* or *Pseudomonas*. Not active

on fungi. Bacteriostatic (1, 5, 11, 15, 33). Cross-resistance with the other tetracyclines and some with chloramphenicol. Active on *Endamoeba histolytica* (with or without associated bacteria), *Colpoda platynematum*, and *Tetrahymena pyriformis* (5, 46, 53, 79). Inhibits the killer action of *Paramecium aurelia* (var. 4, stock 51 killer, mating type VII) (31). Certain concentrations stimulatory to some protozoa (53), *Chlorella* (101), and some yeasts (59). Active on pleuropneumonia-like organism (PPLO) strains at 0.16 to 0.63 μg per ml (30, 32). *In vivo:* Active in various animals on *Streptococcus hemolyticus*, *S. pyogenes*, *D. pneumoniae*, *Staph. aureus*, *B. anthracis*, *K. pneumoniae*, *Pasteurella tularensis*, *P. multocida*, *Sal. typhosa*, *Sal. choleraesuis*, *Sal. enteritidis*, *Sal. newport*, *Sal. typhimurium*, *H. influenzae*, *H. pertussis*, *Br. melitensis*, *Bartonella muris*, *Listerella monocytogenes*, *Spironema duttoni*, *Borrel'a recurrentis*, *B. novyi*, *V. comma*, *Clostridium septicum*, *Cl. tetani*, and *M. tuberculosis*. Moderately active on *Erysipelothrix rhusiopathiae* and *Sal. gallinarum* (1, 5, 6–9, 11, 13, 20, 33, 34, 46, 62). Active on *Endamoeba histolytica* (rats and dogs) (94), *Plasmodium gallinaceum* (chicks), *P. cathemerium* (canaries), *P. berghei* (mice) (35), and *Eperythrozoon coccoides* (mice) (44). Limited activity on toxoplasmosis (rabbit and mouse) (78). Active on arthritis-producing PPLO (L_4 strain) in rats (12). Active on *Rickettsia tsutsugamushi*, *R. prowazekii*, *R. akari*, *R. rickettsii*, *R. burneti*, and *Neorickettsia helmintheca* (5, 77). Has a selective toxicity on the growth of coliphage T2 (102). Active on the agents of primary atypical pneumonia, infectious synovitis (chickens), granuloma inguinale, and lymphogranuloma venereum (5, 95), as well as the mouse and feline pneumonitis and sheep abortion organisms (16, 21, 68). Active on Rous sarcoma cells and infective extracts in contact tests (42). A minimal tolerated dose of 150 mg per kg moderately inhibits sarcoma 180 and carcinoma 1025 (mice) (87). Not active on viruses (10, 47). *Role in nutrition:* (See chlortetracycline for general discussion.) Added to the diet, oxytetracycline increases growth rate of: rats (on a nutritionally deficient diet) (70); chicks (28); germ-free chicks (71); pheasant chicks (63); turkey poults (25); dairy calves (on a limited milk diet) (75); steers (73); weanling pigs (72); human infants (slight effect) (106); but not rabbits (64). *Improves:* Feed efficiency; egg production and egg hatchability in laying hens (41, 88); feed consumption and efficiency in calves (limited milk diet) (75). *Increases:* biotin, folic acid, and vitamin B_{12} in the cecal contents of normal, but not germ-free birds (71); nicotinic acid concentration in the liver of cats (74); and blood sugar levels in dairy calves (76). *Plants:* Oxytetracycline is widely used in combination with streptomycin in controlling certain plant diseases. Alone, it is active on fire blight (*Erwinia amylovora*) of apples (51), bacterial spot (*Xanthomonas pruni*) of peach trees (52), Stewart's corn wilt (*Bacterium stewartii*) (89), leaf blight of corn (*B. carotovorum* f. *zeae*) (92), cucumber wilt (*E. tracheiphila*) (90), and crown-gall (*Agrobacterium tumefaciens*) of cherry (103) and geranium (60). Active on crown-gall tissue (rose) (82). Like chlortetracycline, oxytetracycline has been reported to have activity on certain plant diseases caused by fungi, in spite of its known lack of activity *in vitro* against these organisms. These diseases include leaf spot phase (*Helminthosporium vagans*) of Kentucky blue grass (97) and downy mildew of cucumber (*Pseudoperonospora cubensis*) (98). Oxytetracycline has a vitamin-sparing action (B_1) on virus tumor tissue of *Rumex acetosa* (48). It stimulates growth of young corn seedlings, sorrel (43), wheat roots (29), radishes, cucumbers (48), and a spinach-like plant (55). Stimulates germination, sprout and top growth of oats, and germination and top growth of Madrid sweet clover (55). *Miscellaneous:* Applied to fallow plots, increases bacterial and actinomycete counts, but decreases fungal population. Increases nodulation of sweet clover roots. Nitrates accumulate more rapidly in uncropped, antibiotic-treated soils than in water-treated soils. This effect is reversed in cropped, antibiotic-treated soils (55). Stimulates spermatogenesis in the rat (45).

Toxicity: Animals: Base: LD_{50} (mice) >3500 mg per kg subcutaneously, >5200 mg per kg orally (3), and 100 mg per kg intravenously (15). *Hydrochloride* (expressed as mg of base per kg): LD_{50} (mice) 178 to 192 mg per kg intravenously, 600 to 892 mg per kg subcutaneously, and 6690 to 7200 mg per kg orally (3, 4, 15). LD_{50} (rats) 280 mg per kg intravenously (3). LD_{50} (10-day-old chick embryo) 10.9 mg, via allantoic (105). Highly toxic to guinea pigs, producing toxic symptoms at doses as low as 2.5 mg, orally or intramuscularly (7). *Sulfate:* LD_{50} (mice) 165 mg per kg intravenously, and 600 mg per kg subcutaneously. *Na salt* (pH 8.5): LD_{50} (mice) 150 to 175 mg per kg intravenously; 270 to 300 mg per kg subcutaneously, and 4410 mg per kg orally (3, 4). *Plants:* Inhibits root growth (percentage of control) of the following plants at 100 ppm; mustard (34 per cent), red clover (39 per cent), cucumber (19 per cent), and wheat (38 per cent) (80). Toxic to root growth of *Lupinus albus*

at 1:400,000 (22). Treatment of seed slows growth and inhibits chlorophyll formation in corn salad (*Valerianella locusta*) at 2.0^{-5}, leek at 10^{-5}, lentil at 10^{-6}, and radish at 10^{-4}. In *Cynara cardunculus* (cardoon), root formation is inhibited at 2.10^{-4}, chlorophyll formation at 10^{-5}; but at 2.10^{-4}, no effect on growth. Has a greater toxic effect on young plants than on seeds, increasing flavonoid pigment and anthocyanin concentration, and decreasing chlorophyll, β carotenoids, and carotene (81). Addition of sodium potassium chlorophyll to 500 ppm sprays prevents chlorotic symptoms in plants (104). *Cells:* Least injurious doses for human skin and cord cells of the chick embryo in tissue culture are 25 to 50 and 124 μg per ml, respectively (61). Highest concentration permitting epithelial cell migration in tissue culture is 200 μg per ml (107). Minimal concentration causing mitosis inhibition in HeLa cells is 12.5 μg per ml (99). Inhibits bacteria-free crown-gall tissue at 20 μg per ml, but not normal tissue (82). Toxic to *Allium cepa* root cells at 100 ppm in 12 hours (26).

Utilization: Clinically useful in infections caused by gram-positive and gram-negative bacteria, amoebae, spirochaetes, rickettsiae, and psittacosis group (93).

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Paromomycin

Produced by: Streptomyces rimosus f. *paromomycinus* (1).

Synonyms: Humatin, humycin, antibiotic C 1488. Similar to catenulin.

Method of extraction: Culture-broth adjusted to pH 2.0 and filtered. Filtrate chromatographed on IRC-50 and eluted with 0.5 *N* HCl. Active fractions neutralized to pH 6.0, filtered, then concentrated *in vacuo*. Adsorbed on Darco G-60-diatomaceous earth mixture (65:50 by weight) and eluted with water, 1 per cent aqueous acetone, and 10 per cent aqueous acetone. Eluates concentrated and lyophilized. Reprecipitated from methanol with acetone.

Chemical and physical properties: Basic, amorphous, white substance. Very soluble in water; moderately soluble in methanol, sparingly soluble in absolute ethanol. $[\alpha]_D^{25} = +64°$ (c = 1 per cent in water). Positive ninhydrin and Elson-Morgan tests. Negative maltol and Sakaguchi tests. Infrared spectrum given in reference 1. Stable. C = 45.17%; H = 7.44%; N = 10.35%. *Hydrochloride:* pK_a = 6.3 and 8.35. Structure said possibly to resemble neomycin (2). Acid hydroiysis product is a crystalline basic substance which is biologically inactive. $C_{12}H_{25}O_7N_3$. Structure given below (5).

Biological activity: Active on gram-positive and gram-negative bacteria, including mycobacteria. Not active on *D. pneumoniae, Clostridium perfringens,* or *Ps. aeruginosa.* Active on *Endamoeba histolytica* (1). Cross-resistance with neomycin and kanamycin (2). Shows essentially the same activity as neomycin and kanamycin *in vitro* against *Staph. aureus* and the *Enterobacteriaceae* (2). Active on amoebic infections in dogs and rats (3). Active on murine leprosy (4).

Toxicity: Reputedly nontoxic in topical application (1). Nephrotoxic. Limited absorption from the intestinal tract (3).

Utilization: Amoebic dysentery (3).

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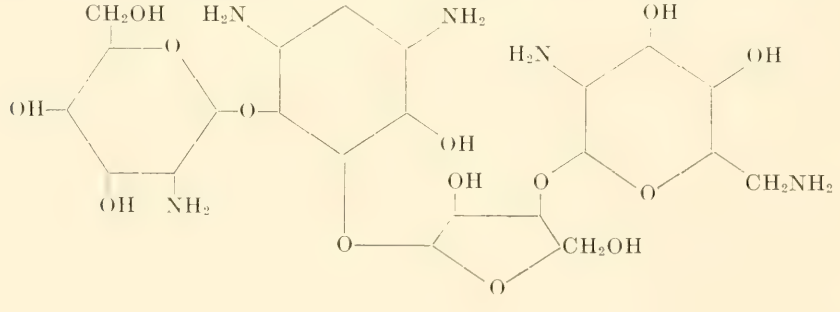
Pentaene Antifungal Antibiotic I

Produced by: Streptomyces lavendulae. This culture also produces streptothricin.

Synonym: Belongs to the eurocidin-like group.

Method of extraction: Broth adjusted to pH 2.5, stirred with Hyflo Filter-Cel, and filtered. Cake adjusted to pH 8 to 9, then extracted with water-saturated n-butanol. Butanol evaporated to dryness *in vacuo*, then resuspended in tert-butanol-water (4:1) for lyophilization. Components separated by countercurrent distribution (ethyl acetate-pyridine-water, 6.5:3.5:8.3).



Chemical and physical properties: Bright yellow powder. Pentaene. Contains two closely related components. Soluble in methanol, ethanol, n-butanol, tert-butanol-water (volume per volume), ethylene glycol, monomethyl ether, N-N-dimethyl sulfoxide, and pyridine. Partially soluble in tert-butanol, water, and chloroform. Insoluble in acetone, benzene, and toluene. Ultraviolet absorption spectrum maxima of both are at about (298), 317, 333, and 350 mμ (methanol) or 302 to 308, 322.5, 338 ($E^{1\%}_{1\,cm}$ 388), and 356 mμ (pyridine). Rf values of two components are 0.55 and 0.85 by ascending paper chromatography (methanol-butanol-water, 1:10:10).

Biological activity: Active on yeasts, fungi, and certain gram-positive bacteria and mycobacteria. Very active on *Coccidioides immitis*.

Reference: 1. Steinman, I. D. Thesis, Rutgers University, 1958.

Pentaene Antifungal Antibiotic II

Produced by: Streptomyces sp.

Chemical and physical properties: Amphoteric pentaene. Yellow substance. Believed to contain a carbohydrate moiety. Approximately one half the nitrogen present is amino N.

Biological activity: Active on fungi. Active at high concentrations on gram-positive bacteria. Hemolytic in high concentrations. Activity affected by serum.

Toxicity: Very toxic for mice.

Reference: 1. Brown, R. and Hazen, E. L. Abstr. Communs. Symposium on Antibiotics, Prague 4–5, 1959.

Pentamycin

Produced by: Streptomyces penticus resembling *S. rubrireticuli* (1), *S. pentaticus* (2).

Synonyms: Related to fungichromin, filipin, and moldcidin B.

Method of extraction: Mycelium extracted with 90 per cent methanol, then 80 per cent methanol. Extracts concentrated *in vacuo*; a gelatinous precipitate forms on standing at room temperature. Dissolved in hot methanol; solution concentrated *in vacuo* until a brownish red precipitate appears. Filtration while hot followed by concentration of filtrate to ½ volume. Crystals precipitate on standing. Recrystallization from methanol (1).

Chemical and physical properties: Pentaene: Pale yellow needles; m.p. 236–237°C (decomposition). Very soluble in pyridine and dimethylformamide. Moderately to slightly soluble in methanol, ethanol, isopropanol, butanol, methyl Cellosolve, acetone, and ethylene glycol. Nearly insoluble in water, ether, ethyl acetate, amyl acetate, benzene, chloroform, and carbon disulfide. Ultraviolet absorption spectrum maxima at 322 ($E^{1\%}_{1\,cm}$ 899), 338 ($E^{1\%}_{1\,cm}$ 1450), and 356 mμ ($E^{1\%}_{1\,cm}$ 1500), and a shoulder at 308 to 313 mμ. Infrared spectrum given in reference 1. Gives a deep purple color in H_2SO_4. Reduces Tollen's reagent very slowly, and decolorizes Br water. Negative Fehling and $FeCl_3$ tests (1). Very stable at pH 4 to 6 at 37°C; unstable at pH 1 and pH 10. C = 61.5%; H = 8.4%. Chromophoric moiety is 2-methyl-2,4, 6,8,10-dodecapentaenedial, as in fungichromin (2).

Biological activity: Active at 0.2 to 4 μg per ml on fungi and yeasts. No activity on bacteria (1). Inhibits Ridgeway osteogenic sarcoma (3).

Toxicity: LD_{50} (mice) 33.3 mg per kg intraperitoneally, and 1624 mg per kg orally (1).

References:

1. Umezawa, S. *et al.* J. Antibiotics (Japan) **11A:** 26–29, 1958.
2. Umezawa, S. *et al.* J. Antibiotics (Japan) **11A:** 273, 1958.
3. Sugiura, K. *et al.* Cancer Research **18:** 66–77, 1958.
4. Ogawa, H. *et al.* J. Antibiotics (Japan) **13A:** 353–355, 1960.

Perimycin

Produced by: Streptomyces coelicolor var. *aminophilus* (1).

Synonyms: Antibiotic 1968 (2), antibiotic NC 1968 (1).

Method of extraction: Extraction of the mycelium with methanol at a slightly alkaline pH (1). Crude extracts can be purified by dissolving in a hot methanol-ethyl acetate (5:1) mixture. Cooling causes precipitation. Precipitate centrifuged and washed with ethyl ether. Wet precipitate dissolved in n-butanol (1:1), and pH adjusted to 10 to 11. Butanol layer separated, washed with water until neutral, and concentrated *in vacuo*. Precipitation occurs during concentration and is completed by adding ethyl ether to the concentrate. Further purification by countercurrent distribution in ethyl acetate-pyridine-water (6.5:3.5:8.3; partition coefficient, 2.17).

Chemical and physical properties: Heptaene. Light-absorption maxima at 361, 383, and 406 mμ. $E^{1\%}_{1\,cm}$ at 383 mμ = 1000 in methanol. Decomposes slowly upon heating. Insoluble in water, petroleum ether, ethyl acetate, and benzene. Soluble in aqueous lower alcohols, pyridine, tetrahydrofuran, acetone, and dioxane. Soluble in hot

methanol and in dimethylformamide, dimethyl sulfoxide, and lower fatty acids. Infrared data given in reference 3. $C_{47}H_{75}O_{14}N_2$. The molecule contains four C—CH_3 groups, no carboxylic group, and two free amino groups. One of the amino groups is in a glycosidically linked amino sugar which is different from mycosamine. The other amino group is in *p*-aminophenylacetone, which is linked to the rest of the molecule through an aldol system (3).

Biological activity: Active on yeasts and filamentous fungi (2, 3). Can be differentiated biologically from trichomycin, candicidin, candidin, and amphotericin B (3). Active in mice on experimental histoplasmosis, sporotrichosis, and moniliasis (4).

References:

1. British Patent 826,792, February 24, 1960.
2. Oswald, E. J. *et al.* Antibiotics Ann. 236–239, 1955–1956.
3. Borowski, E. *et al.* Trans. Conf. antimicrobial agents, Washington 1960.
4. Campbell, C. C. *et al.* Antibiotics Ann. 240–246, 1955–1956.

Phaeofacin

Produced by: Streptomyces phaeofaciens (1).

Method of extraction: I. Broth-filtrate extracted with ethyl acetate at pH 7.0. Extract concentrated *in vacuo*. Syrup dissolved in acetone and cooled to precipitate phaeofacin. II. Broth adjusted to pH 2.0. Precipitate which forms extracted with ethanol or acetone to elute the antibiotic. III. Mycelium extracted with acetone. Extract evaporated *in vacuo* and adjusted to pH 2.0 to precipitate phaeofacin.

Chemical and physical properties: Crystalline substance deliquescing on drying. Soluble in ethyl acetate, ethanol, acetone, and benzene. Slightly soluble in ether and petroleum ether. Insoluble in water. Positive $FeCl_3$ test. Negative biuret, indole, Millon, ninhydrin, Molisch, Tollen, and Sakaguchi tests (1,2).

Biological activity: Active on yeasts and fungi (1). Not active on bacteria (2).

Toxicity: MLD (mice) 200 mg per kg intraperitoneally (1).

References:

1. Maeda, K. *et al.* J. Antibiotics (Japan) **5:** 465, 1952.
2. Maeda, K. *et al.* Japan. J. Med. Sci. & Biol. **5:** 327–339, 1952.

Phagocidin

Produced by: Streptomyces sp. resembling *S. antibioticus* (1).

Method of extraction: Filtered broth extracted with butanol at pH 5.0. Butanol evaporated *in vacuo*. Residue taken up in water and treated with ethyl ether to remove another antibiotic similar to actinomycin. Extraction of the resulting aqueous solution with ethyl acetate, followed by evaporation to dryness *in vacuo*. Purification from a methanol-benzene solution (1:9) by chromatography on alumina. Eluted with methanol-benzene (1:9 and 2:8) and finally methanol (1).

Chemical and physical properties: Yellow-tinged crystals. Acidic substance. Soluble in methanol, ethanol, n-butanol, ethyl and butyl acetates, dichloroethylene, chloroform, acetone, and *N* NaOH. Slightly soluble in dilute alkaline water. Insoluble in benzene, ethyl ether, petroleum ether, and distilled water. Negative ninhydrin, Fehling, $FeCl_3$, Millon, biuret, and Hopkins-Cole tests. Positive Molisch test. Ultraviolet absorption spectrum maxima at 235 ($E^{1\%}_{1\,cm}$ 1200) and 300 mμ ($E^{1\%}_{1\,cm}$ 225) in a 1 per cent methanol solution (1).

Biological activity: No activity on the gram-positive and gram-negative bacteria tested. Active on a variety of phages, including *E. coli* phages T_3, T_5, and T_7, and *Sal. pullorum*, *Sal. enteritidis*, *Sal. paratyphi* B, and *Shigella flexneri* phages. Not active on *E. coli* T_1, T_2, T_4, or T_6, *Sal. paratyphi* A, or *Staph. aureus* phages. At a given concentration, the per cent survival rate of T_3 phage is the same regardless of the concentration of phage particles. However, against a given number of phage particles, the per cent survival rate decreases as the concentration of the antibiotic increases (1). Activity on free phage is greater at 37°C than at 5°C and is irreversible. Inactive on phage adsorbed on host at 37°C, but active on phage adsorbed at 5°C. At 5°C, more active on free phage than phage adsorbed on the host (2).

Toxicity: Mice tolerate 5 mg per kg, but not 20 mg per kg intraperitoneally (1).

Reference: 1. Higo, N. and Hinuma, V. J. Antibiotics (Japan) **9A:** 152–163, 1956.

Phagolessin

Produced by: Streptomyces sp. (1), *S. griseus* (3).

Synonym: Antibiotic A 58.

Method of extraction: Broth adjusted to pH 3.3, neutral lead acetate added to a 1 per cent concentration, and broth filtered. Filtrate adjusted to pH 2.0 and phosphotungstic acid added. Precipitate which forms extracted with 80 per cent ethanol at pH 3.0 to 3.2. Addition of lead acetate to extract precipitates impurities. Chromatography

on alumina. Developed with 80 per cent acidic ethanol (pH 3.0). Eluates concentrated and lyophilized (1).

Chemical and physical properties: Light yellow hygroscopic powder. Soluble in acidic water and methanol. Less soluble in ethanol. Insoluble in acetone, ether, benzene, chloroform, and petroleum ether. Negative $FeCl_3$, biuret, Millon, and ninhydrin tests. Positive Lassaignes test for nitrogen. Reacts with picric acid, Reinecke's salt, and methyl orange. Stable in dry form. In solution most stable at pH 3.0 at 100°C for 5 minutes; irreversibly inactivated at pH 9.0.

Biological activity: Active on *K. pneumoniae* at 3.0 μg per ml. Less, or not active on other bacteria tested, including *E. coli.* and *Staph. aureus* (12 μg per ml), and *Mycobacterium smegmatis* (50 μg per ml) (1). Also active on *Borrelia duttonii*, *Plasmodium berghei*, and *Trypanosoma equiperdum* (3). Has a direct killing action on certain phages: coliphages T_1, T_3, and T_7 (but not T_2, T_{4r}, T_5, or T_6 (2)), *Eberthella typhosa* phage, *Sal. paratyphi* phage, and *Clostridium perfringens* phage (3). Somewhat active on *S. griseus* phage. Active mainly on free phage, but also inhibits certain absorbed phage in the early part of the latent period. Inactivated phage retains ability to be absorbed and kill host cells, but not to multiply (1). Action not affected by increasing the number of phage. Inactivation of free phage inhibited by deoxyribonucleic acid and to a lesser extent by ribonucleic acid (2). Active in tissue culture on Y-SK poliomyelitis virus. Inhibits PR 8 influenza virus but not herpes simplex in eggs (4). Virucidal (*in vitro*) action on vaccinia (L.N. and Nelis strains) and herpes simplex; destroys rabies virus (Brussels strain) on contact; partial virucidal action on poliomyelitis (Theiler and Lansing strains). No *in vitro* action on lymphogranuloma or PR 8 influenza virus (3).

Toxicity: Mice are killed in 72 hours by injections of 31.25 mg per kg intraperitoneally, or 500 mg per kg subcutaneously. Intravenous injection of 500 mg per kg causes immediate death (1). Toxic to mice at therapeutic levels. Not toxic to tissue cultures at 20 mg per ml (3, 4).

References:

1. Asheshov, I. N. *et al.* Antibiotics & Chemotherapy **2:** 366–374, 1952.
2. Hall, E. A. and Asheshov, I. N. J. Gen. Physiol. **37:** 217–230, 1953.
3. Levaditi, C. *et al.* Rev. immunol. **17:** 324–338, 1953.
4. Asheshov, I. N. *et al.* Antibiotics & Chemotherapy **4:** 380–394, 1954.

Phagomycin

Produced by: Streptomyces sp. resembling *S. griscolus.*

Method of extraction: Broth adjusted to pH 5.0 and filtered. Filtrate adjusted to pH 7.0 and passed through an Ionex C cation-exchange resin column. Elution with 80 per cent aqueous acetone containing 0.2 *N* HCl. Active fractions adjusted to pH 7.0, filtered, and readjusted to pH 5.0. Acetone concentrated to dryness under nitrogen. Purification by chromatography on alumina from methanolic solution developed with dry methanol, and chromatography on Darco G-60 plus Dicalite from acidic water.

Chemical and physical properties: Basic substance. Soluble in water and methanol. Less soluble in ethanol and aqueous acetone. Positive ninhydrin test. Negative biuret, xanthoproteic, Sakaguchi, maltol, Molisch, Millon, Fehling, Hopkins-Cole, Tollen, and $FeCl_3$ tests. Stable at acid pH. Unstable at neutrality or at alkaline pH. Ultraviolet absorption spectrum shows end-absorption only. *Reineckate:* m.p. 166–170°C (decomposition). C = 12.38%; H = 6.79%; N = 12.14%; S = 14.34%; Cr = 5.24% (*sic*).

Biological activity: Not active on bacteria, fungi, or protozoa. Active on some bacteriophages, including coliphages T_1, T_3, T_5, and T_7, but not T_2 and T_6 or micrococcus phage. Active on *Sal. enteritidis*, *S. paratyphi* B, and *Shigella flexneri* phages. Antiphage activity against free, but not adsorbed phage.

Toxicity: Mice tolerate about 66 mg per kg intraperitoneally

Reference: 1. Miura, M. J. Antibiotics (Japan) **9A:** 108–112, 1956.

Phagostatin

Produced by: Streptomyces sp. resembling *S. bikiniensis.*

Method of extraction: Ninety per cent of the antibiotic is present in the mycelium and is extracted with 80 per cent aqueous acetone. Broth extracted with butanol. Acetone-extract concentrated *in vacuo* to an aqueous residue. Residue extracted with butanol, this extract combined with the butanol-extract from the broth, and both extracts concentrated *in vacuo* following the addition of water. Watery residue extracted with chloroform. Extract concentrated to dryness. Purification by recrystallization from 50 to 60 per cent aqueous acetone, or chromatography on alumina from chloroform-20 per cent ether, developed with chloroform, ethyl acetate, and acetone, and eluted with acetone. The antibiotic is precipitated from

acetone-eluates in the cold and recrystallized from 50 per cent aqueous acetone.

Chemical and physical properties: Long needles; m.p. 184–190°C (decomposition) with yellowing at 125–130°C. Soluble in chloroform, acetone, ethanol, and n-butanol. Slightly soluble in anhydrous methanol and ethyl acetate. Insoluble in water, benzene, ether, and petroleum ether. Electrophoretic paper chromatography indicates that the antibiotic is neutral or weakly basic. Negative ninhydrin, Fehling, biuret, Molisch, Hopkins-Cole, Millon, Ehrlich (*p*-dimethylbenzaldehyde), and $FeCl_3$ reactions. Thermostable in a wide pH range. No characteristic ultraviolet absorption. Infrared absorption spectrum given in reference 1.

Biological activity: Active on a certain phage-sensitive strain of *E. coli* B and moderately active on *Sal. enteritidis*, but not other salmonellae tested. Moderately active on *B. anthracis*, *Sarcina lutea*, and *Micrococcus citreus*. Not active on *Staph. aureus*, *Shigella sonnei*, *Ps. aeruginosa*, or *Pr. vulgaris*. Prevents plaque formation by T_3 and T_7 coliphages in the presence of host *E. coli* B, but at concentrations slightly lower than those required to inhibit the host. Not active on free phage, and does not prevent absorption of T_3. Action (using T_3 phage) shown not to be on intracellular phage synthesis, but the inhibition of lysis of the host cell, thus preventing release of intracellular phage (2).

References:

1. Hamada, M. J. Antibiotics (Japan) **10A:** 74–79, 1957.
2. Higo, N. Japan. J. Microbiol. **2:** 203–215, 1958.

Phalamycin

Produced by: Streptomyces noursei. This strain is a variant of the nystatin-producer.

Method of extraction: Broth-filtrate frozen, then thawed. First two sevenths of melt, containing most of the antibiotic, extracted with ethyl acetate at pH 7.5 to 8.0. Extract concentrated *in vacuo*. Residue taken up in methanol, cooled, filtered, and concentrated *in vacuo*. Chloroform solution of residue washed with water and concentrated *in vacuo*. Residue washed with ether and extracted with ethyl acetate. Crystallized from acetone. Can also be extracted from mycelium (1, 2).

Chemical and physical properties: Fragile, long, sharp-pointed hexagonal plates. No characteristic melting point; gradually decomposes but does not melt when heated to 285°C. Soluble in lower alcohols, esters, chloroform, and acetone. Slightly soluble in benzene, ether, and water. No characteristic ultraviolet spectrum. Infrared data given in reference 2. Tests indicate the presence of unsaturation, enolic function, and primary or secondary alcoholic function. Negative biuret, ninhydrin, Sakaguchi, Molisch, Millon, and $FeCl_3$ tests, as well as tests for organic base and methyl ketone. Relatively stable from pH 2.5 to 7.0. C = 53.5%; H = 5.45%; N = 13.65%; S = 5.11% (4); or C = 50.10%; H = 4.78%; S = 3.67%; N = 14.98%. $C_{36}H_{41}O_{14}N_3S$ (2). Does not contain the same amino acids as bryamycin (4).

Biological activity: Active on gram-positive bacteria, including mycobacteria and nocardiae. Inactive on gram-negative bacteria and fungi (1). Tests with pure material failed to confirm earlier reports on *in vivo* activity on *Streptococcus hemolyticus* (Group A) infections in mice (3).

Toxicity: Mice tolerate >115.7 mg per kg subcutaneously, and >29 mg per kg intraperitoneally, of the crude material. An oral dose of about 1 gm per kg of purified phalamycin tolerated by mice, possibly as a result of poor absorption (3).

References:

1. Brown, R. and Hazen, E. L. Antibiotics & Chemotherapy **3:** 818–821, 1953.
2. Brown, R. Ann. Rept. Div. Lab. and Research, N.Y. State Dept. Health. 18–19, 1956.
3. Hazen, E. L. Ann. Rept. Div. Lab. and Research, N. Y. State Dept. Health 19–20, 1956.
4. British Patent 790,521, February 12, 1958.

Phleomycin

Produced by: Streptomyces verticillus (3). Broth contains two other antibiotics active on *B. subtilis* but inactive on *Staph. aureus* or *Mycobacterium* 607 (4).

Method of extraction: Adsorbed from broth at pH 7.0 onto Amberlite IRC-50 (Na^+ phase) and eluted with 10 per cent NaCl. Active fractions pooled and concentrated *in vacuo*. Concentrate cooled, filtered to remove the salts, and acetone added to precipitate phleomycin. Reprecipitated from a concentrated methanolic solution by addition of ether. Purification by chromatography on alumina with 80 per cent methanol as solvent and developer. Prepared as a reineckate (1, 3). Reineckate washed with ammonia-acetone to give base. Purified by chromatography on alumina (water-methanol, 1:2 to 3) (4).

Chemical and physical properties: Hydrochloride: White, amorphous, hygroscopic powder. Soluble in water, dilute ethanol, and methanol. Slightly soluble in ethanol. Insoluble in acetone, ether,

butyl and ethyl acetate, chloroform, benzene, and petroleum ether. Ultraviolet absorption spectrum shows only end-absorption. Positive ninhydrin (in pyridine) and glucosamine tests. Negative ninhydrin (in water), Sakaguchi, Tollen, Fehling, biuret, maltol, and $FeCl_3$ tests. No S or halogen. *Reineckate:* Red plates; m.p. 195–197°C (decomposition). Infrared absorption spectra of the hydrochloride and reineckate given in reference 1. Unstable in weakly acid solution. Most stable at pH 7 to 9. Possibly composed of a peptide and a carbohydrate moiety (1, 3). Hydrolysis products (concentrated HCl) include two ninhydrin-positive substances (Rf values given in reference 3); product with 40 per cent H_2SO_4 may be furfural (3). *Base:* Cobalt-blue amorphous powder. Soluble in water; slightly soluble in methanol; insoluble in other organic solvents. Ultraviolet absorption spectrum maxima at 244 ($E^{1\%}_{1\,cm}$ 135) and 295 to 300 mμ ($E^{1\%}_{1\,cm}$ 47). Positive ninhydrin and diazo reactions. Negative Fehling, Tollen, Sakaguchi, and Molisch tests. $C_{53}H_{91}N_{17}O_{32}Cu$ (4). *Copper-free phleomycin:* White to pale green amorphous powder. Copper easily reintroduced to give phleomycin (4).

Biological activity: Weakly active on bacteria. Active against *M. phlei* at <0.3 μg per ml. Not active on fungi. Partial cross-resistance with kanamycin when *E. coli* is used, but not with *Mycobacterium* 607 (2). Copper-free phleomycin is as active as phleomycin, except against mycobacteria.

Toxicity: Hydrochloride: LD_{50} (mice) 250 to 500 mg per kg intravenously. No delayed toxicity noted (1). *Phleomycin:* LD_{50} 40 to 50 mg per kg (no route or animal given) (4). *Copper-free phleomycin:* LD_{50} 150 mg per k8 (no route or animal given) (4).

References:

1. Maeda, K. *et al.* J. Antibiotics (Japan) **9A:** 82–85, 1956.
2. Umezawa, H. Ann. N. Y. Acad. Sci. **76:** 20–26, 1958.
3. Japanese Patent 2598, April 17, 1959.
4. Takita, T. *et al.* J. Antibiotics (Japan) **12A:** 111, 1959.

Phthiomycin

Produced by: Streptomyces luteochromogenes (1).

Synonym: Closely related to viomycin (1).

Method of extraction: Adsorption on cation exchange resins, elution with 0.5 to 1.0 *N* HCl. Precipitation of the crude hydrochloride by methanol (1).

Chemical and physical properties: Basic substance, white. Positive biuret, Sakaguchi, and ninhydrin test. Negative Molisch, maltol, and $FeCl_3$ tests. Ultraviolet light-absorption maxima at 268 (0.1 *N* HCl) and 282 mμ (0.1 *N* NaOH) (1, 3). $[\alpha]_D^{150} = -11°$. Forms a water-soluble hydrochloride and sulfate (3).

Biological activity: Active in concentrations of 6 to 25 μg per ml against gram-positive bacteria and mycobacteria. No activity against fungi. Some activity against the tubercle bacillus in mice.

Toxicity: LD_{50} (mice) 600 mg per kg intravenously.

References:

1. Maeda, K. *et al.* J. Antibiotics (Japan) **6A:** 183–185, 1953.
2. Miyamoto, Y. and Maeda, K. J. Antibiotics (Japan) **7A:** 17–20, 1954.
3. Umezawa, H. *et al.* Japanese Patent 3096, May 9, 1955.

Phytoactin

Produced by: Streptomyces sp.

Chemical and physical properties: Polypeptide. Soluble in methanol, ethanol, 1-butanol, chloroform, acetone, methyl isobutyl ketone, and other organic solvents. Slightly soluble in ether and water. Insoluble in petroleum ether. Heat-stable. Not digested by pepsin, trypsin, or proteases. Dialyzes through cellophane into aqueous methanol. Acid hydrolysates contain valine, α-alanine, proline, leucine (or isoleucine), arginine, glycine, and serine (1).

Biological activity: Active on gram-positive bacteria and fungi. Control of tomato early blight (greenhouse studies), tomato late blight, and bean rust (1). Active in field trials on powdery mildew (*Podosphaera leucotricha*) of pear (2).

Toxicity: Not toxic to tomato or bean plants at therapeutic levels (1).

References:

1. Ziffer, J. S. *et al.* Phytopathology **47:** 539, 1957.
2. Sprague R. Plant Disease Reptr. **42:** 1208–1209, 1958.

Phytostreptin

Produced by: Mutant of phytoactin-producing *Streptomyces* sp.

Chemical and physical properties: Polypeptide. Soluble in water, methanol, ethanol, 1-butanol, chloroform, acetone, methyl isobutyl ketone, and other organic solvents. Slightly soluble in ether. Insoluble in petroleum ether. Heat-stable. Not digested by pepsin, trypsin, or proteases. Dialyzes through cellophane into water. Acid hydrolysates contain same amino acids as phytoactin (1).

Biological activity: Active on gram-positive

bacteria and fungi. Active on tomato early blight (greenhouse studies), tomato late blight, and bean rust (1). Active on powdery mildew of pear (*Podosphaera leucotricha*) in the greenhouse (2).

Toxicity: Not toxic to tomato and bean plants at therapeutic levels (1).

References:

1. Ziffer, J. S. *et al.* Phytopathology **47:** 539, 1957.
2. Sprague, R. Plant Disease Reptr. **42:** 1208–1209, 1958.

Picromycin

Produced by: Streptomyces felleus strains (2, 8, 18), *Streptomyces* spp. (2, 7, 16).

Synonyms: Argomycin (12), antibiotic BU 277 (18). Similar or identical to proactinomycin A (13) and amaromycin (11). Griseomycin is isomeric with picromycin (13). Differs from methymycin only in point of attachment of the desosamine to the lactone ring.

Method of extraction: Concentrated broth, or an aqueous solution of the residue of a concentrated ethyl acetate extract of the broth, is extracted at pH 9.0 with ether. Ether concentrated and re-extracted into 0.1 *N* HCl at pH 1 to 2. Adjusted to pH 9 to 10 with Na_2CO_3, filtered, and extracted with ether. Ether taken to dryness *in vacuo*. Crystallized from acetone, boiling methanol, or ethanol, or purified by salt conversion. Further purification by fractional distribution and chromatography (1, 2, 16).

Chemical and physical properties: Macrolide. Colorless, bitter-tasting crystals resembling coffin-covers; m.p. 169–170°C. Very soluble in acetone, benzene, chloroform, ethyl acetate, dioxane, and dilute acids. Moderately soluble in ether and cold methanol. Scarcely soluble in water, petroleum ether, or carbon disulfide. Ultraviolet absorption spectrum maxima at 222 (log ϵ = 4.02) and 293 mμ (log ϵ = 1.83) (15) or at 225 mμ (log ϵ = 3.97) (16). Infrared spectrum given in reference 16. $[\alpha]_D^{24}$ = −50.2° (c = 6.3 per cent in chloroform) and +8.2° (c = 3.5 per cent in ethanol). Orange-red solution in concentrated H_2SO_4 gives only end-absorption of ultraviolet light. This color fades on dilution, giving a precipitate. Positive $KMnO_4$ test in glacial acetic acid. Negative bromine, ninhydrin, and *p*-nitrophenylhydrazine tests. Warming with dilute acid or alkali destroys biological activity. C = 63.86%; H = 9.02%; N = 2.92%; N—CH_3 = 4.0%; active H = 0.59%; CH_3 = 16.2%. Molecular weight, 458. $C_{25}H_{43}O_7N$. *Picrate:* Yellow. Amorphous hydrochloride and picrolonate (1, 2, 5, 8, 15). *Quaternary salt with methyl iodide:* $C_{29}H_{51}NO_8I$; m.p. 193–195° C $[\alpha]_D$ = +24.7° (ethanol) (13). Mild acid hydrolysis products include: (a) kromycin ("methynolide" of methymycin), $C_{17}H_{28}O_4$, a biologically inactive, tasteless product. On oxidation with $KMnO_4$ in acetone, kromycin yields the lactone of β-hydroxy-α-α',γ-trimethylpimelic acid. This lactone is also formed from methymycin and narbomycin; (b) picrocin (desosamine). *Hydrochloride:* Colorless, water-insoluble needles; m.p. 189–191°C (decomposition). $[\alpha]_D^{20}$ = +49.5° (water). $C_8H_{17}O_3N \cdot HCl$ (9,15). Complete structure of picromycin (15, 17) given in Chapter 6.

Biological activity: Active on gram-positive bacteria, but not on gram-negative bacteria except certain strains of *Neisseria* and *Pasteurella*. Active on mycobacteria. No activity on filamentous fungi or yeasts (5, 7). Reputed to be active on gram-positive bacteria *in vivo* (16), but has no activity on coccal or *Miyagawanella bronchopneumoniae* infections in mice (7).

Toxicity: MLD (mice) 0.1 to 0.7 gm per kg intravenously (8). Maximal tolerated dose (mice) 100 mg per kg per day subcutaneously, and 250 mg per kg per day intraperitoneally (7).

Utilization: Favorable effect on bacterial skin infections (3).

References:

1. Brockmann, H. and Henkel, W. Naturwissenchaften **37:** 138–139, 1950.
2. Brockmann, H. and Henkel, W. Chem. Ber. **84:** 284–288, 1951.
3. Suhren, O. Med. klin. (Munich) **46:** 722, 1951.
4. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
5. Brockmann, H. *et al.* Chem Ber. **85:** 426–433, 1952.
6. Brockmann, H. and Strufe, R. Chem Ber. **86:** 876–884, 1953.
7. Brodersen, R. *et al.* Acta Pharmacol. Toxicol. **9:** 255–258, 1953.
8. Brockmann, H. and Bohne, A. U. S. Patent 2,693,433, November 2, 1954.
9. Brockmann, H. *et al.* Chem. Ber. **87:** 856–865, 1954.
10. Brockmann, H. and Oster, R. Naturwissenschaften **42:** 155, 1955.
11. Hata, T. *et al.* J. Antibiotics (Japan) **8A:** 9–14, 1955.
12. Shibata, M. *et al.* Quoted in reference 11.
13. DeSomer, P. Giorn. microbiol. **2:** 216–232, 1956.
14. Anliker, R. *et al.* Helv. Chim. Acta **39:** 1785–1790, 1956.

15. Brockmann, H. and Oster, R. Chem. Ber. **90:** 605–617, 1957.
16. Anliker, R. and Gubler, K. Helv. Chim. Acta **40:** 119–129, 1957.
17. Anliker, R. and Gubler, K. Helv. Chim. Acta **40:** 1768–1772, 1957.

Pimaricin

Produced by: Streptomyces natalensis.

Method of extraction: Extraction of mycelium with lower alcohols, glycols, or formamide and its derivatives. Partial evaporation of such extracts followed by addition of water gives a crystalline precipitate (pimaricin). Further purification by recrystallization from hot methanol, or by reisolation from its organic or inorganic salts (1).

Chemical and physical properties: Tetraenic macrolide. Colorless. Decomposes at about 200°C. Soluble in methylpyrrolidone, methyl Cellosolve plus 2 per cent $CaCl_2$, and dilute acids and alkalies. Slightly soluble in methanol, propylene glycol, diethylene glycol, and formamide. Practically insoluble in water. λ_{max} 279, 290, 303, and 318. $[\alpha]_D = +250°$. Positive tests for primary amino groups (Van Slyke), carboxyl, and keto groups. Positive iodoform test in aqueous bicarbonate solution. Stable when dry or in solution at pH 5 to 7 in water. Photosensitive, subject to autoxidation, relatively thermostable. Na-K chlorophyllin and other substances which strongly absorb ultraviolet light in the range of 300 to 400 mμ prevent loss of activity of pimaricin in ultraviolet light and in the air (5). $C_{34}H_{49}NO_{14}$: C = 58.53 ±0.32%; H = 7.32 ± 0.17%; N = 2.12 ± 0.14%; C—CH_3 = 1.43%. Hydrolysis with methanolic HCl gives mycosamine. Alkaline hydrolysis gives 13-hydroxy-2,4,6,8,10-tetradecapentaeneal. This compound has the following characteristics: orange crystals; m.p. 124–128°C. Ultraviolet absorption spectrum maximum at 375 mμ (methanol). Positive Fehling test (1, 3, 4). Complete structure of pimaricin given in Chapter 6. *Na salt:* More water-soluble than pimaricin (5).

Biological activity: Active on fungi and yeasts, both pathogenic and saprophytic. Active on *Trichomonas vaginalis.* Fungicidal. Active over a wide pH range. Suppresses intestinal overgrowth by yeasts after tetracycline administration in rats and mice (2).

Toxicity: LD_{50} (rat) 1500 mg per kg orally, 650 mg per kg intraperitoneally, 2000 mg per kg intramuscularly, and 5000 mg per kg subcutaneously. LD_{50} (mice) 1500 mg per kg orally. LD_{50} (guinea pig) 450 mg per kg orally. No toxic effects on rats fed 50 to 70 mg per kg for 5 to 10 weeks, but 150 mg per kg inhibit growth.

References:

1. Struyk, A. P. *et al.* Antibiotics Ann. 878–885, 1957–1958.
2. Manten, A. and Hoogerheide, J. C. Antibiotics & Chemotherapy **8:** 381–386, 1958.
3. Patrick, J. B. *et al.* J. Am. Chem. Soc. **80:** 6688–6689, 1958.
4. Dutch Patent 87,323, January 15, 1958.
5. Dekker, J. and Ark, P. A. Antibiotics & Chemotherapy **9:** 327–332, 1959.

Pleocidins

Produced by: Streptomyces sp. resembling *S. lavendulae* (1).

Synonyms: Streptothricin-like antibiotic. Pleocidin I appears to be streptothricin (see below) (3). See also streptothricin-like antibiotics.

Method of extraction: I. Extracted like streptothricin. II. Broth treated with oxalate to remove Ca^{++} ions, then adsorbed on IRC-50 at pH 8.0 and eluted with 10 per cent aqueous HCl. Freed of excess acid by passage over IR4B, then lyophilized. Purified by helianthate cyclization (1). III. Purification of the complex by chromatography on Darco G-60-Hyflo Super-Cel (1:2) from aqueous solution. Developed first with distilled water to give Fraction A, then with methanol-1 *N* HCl-water (15:2:8) to give B. Rf values of the components contained in Fractions A and B will be found in Table 43 under "streptothricin-like antibiotics." Lyophilized fractions taken up in methanol and precipitated with acetone. Complex further resolved by chromatography on cellulose with water as solvent and 1-propanol-pyridine-acetic acid-water (15:10:3:12) as developer. Ether added to the effluent fractions, and the antibiotic-containing lower phases lyophilized. Pleocidin I

TABLE 42

Biological activity of pleocidins (Horowitz, 1957)

Antibiotic	Minimum inhibitory concentration			
	B. subtilis	*E. coli*	*S. cerevisiae*	*C. tropicalis*
	μg/ml			
Pleocidin complex	1.3	2.3	5.9	5.0
Pleocidin I	0.55	2.5	67	67
Streptothricin (commercial)	0.55	2.5	67	67
Pleocidin II	0.83	2.8	3.7	3.7
Pleocidin III	1.4	5.0	7.7	7.7
Pleocidin IV	1.4	14	13	20

purified from the appropriate fraction by treatment with oleic acid-treated Darco G-60 and acetone, and precipitation with absolute methanol and acetone. Pleocidin II obtained by treatment of the appropriate fraction with IR-45 (Cl^- phase), lyophilization, and chromatography on cellulose as above. Active fractions again lyophilized. Solid taken up in methanol-3 *N* HCl, treated with Darco G-60, and precipitated with acetone. Pleocidin III separated from contaminating traces of I and II by helianthate cyclization. A fourth component, pleocidin IV, is not completely purified (3).

Chemical and physical properties: Complex hydrochloride: White hygroscopic powder. Soluble in water, methanol, and ethanol. Most stable at pH 4 to 6 in aqueous solution. Rf values of the components of the complex given in Table 43 under "streptothricin-like antibiotics." *Pleocidin I*: Rf = 0.50 by circular paper chromatography with the system 1-propanol-pyridine-acetic acid-water (15:10:3:12). Apparently identical to streptothricin. *Pleocidin II:* Hydrochloride: Faintly buff-colored substance. $[\alpha]_D^{25} = -38°$ (c = 1.1562 per cent in water). Infrared spectrum given in reference 3. Rf = 0.43 in system under I. *Pleocidin III:* Hydrochloride: Contains traces of pleocidin I. $[\alpha]_D^{25} = -23.6°$ (c = 0.9428 per cent in water). Rf = 0.35 in above system. *Pleocidin IV:* Rf = 0.26 in above system. The infrared spectra of the various pleocidins do not differ qualitatively (3).

Biological activity: (See Table 42 and reference 3). Like streptothricin, pleocidin is actively translocated by tobacco, tomato, and bean plants (2). Unlike streptothricin, pleocidin has anthelmintic activity similar to mycothricin (3). Active on *Chlorella pyrenoidosa* at 5 μg per ml (4).

Toxicity: LD_{50} (mice) 3 mg per kg intraperitoneally, about 120 mg per kg orally (1).

References:

1. Charney, J. *et al.* Antibiotics & Chemotherapy **2:** 307–310, 1952.
2. Gray, R. A. Suppl. Proc. Plant Physiol. Meetings Pl. Physiol. **30:** vi, 1955.
3. Horowitz, M. I. Thesis, Rutgers University, 1957.
4. Tomisek, A. *et al.* Plant Physiol. **32:** 7–10, 1957.

Pleomycin

Produced by: Streptomyces pleofaciens.

Method of extraction: Filtered broth extracted with ½ volume of ethyl acetate at pH 2.0. Ethyl acetate washed with ¼ volume of water, then concentrated to ¼ to ⅛ volume. Ethyl acetate back-extracted with ¾ volume of 1 per cent $NaHCO_3$. After adjustment of the pH of the aqueous extract to 2.0, extraction with ether. Ether evaporated *in vacuo*. Residue dissolved in ethanol. Crystalline pleomycin precipitates upon cooling.

Chemical and physical properties: Acid, crystallizing as glistening, colorless, rectangular plates; m.p. 235°C (uncorrected). Sublimes at 200°C. Soluble in methanol, ethanol, ethyl acetate, diethyl ether, and benzene. Alkali salts are readily soluble in water. C = 54.63%; H = 3.94%; O (by difference) = 41.43%. Molecular weight, 303. Empirical formula: $C_{14}H_{12}O_8$. Maximal absorption in ultraviolet light at 270 ($E_{1\,cm}^{1\%}$ 4500) and at 330 to 340 mμ ($E_{1\,cm}^{1\%}$ 350). Maximal absorption in infrared light (Nujol) at 2.90, 3.23, 3.43, 3.54, 6.29, 6.59, 6.85, 7.08, 7.28, 7.44, 7.80, 7.88, 8.20, 8.48, 8.60, 9.60, 10.16, 12.24, 12.62, 12.92, 13.80, and 15.07 mμ.

Biological activity: Active *in vitro* against various gram-positive and gram-negative bacteria, including *Staph. aureus*, *B. subtilis*, *Pr. vulgaris*, *Ps. aeruginosa*, and *M. tuberculosis* H37Rv at the level of 6.25 to 12.5 μg per ml. Inactive against *Sacch. cerevisiae* and *A. niger*.

Toxicity: LD_{50} (mice) 35 mg per kg intraperitoneally. A single application of 1 mg per ml in a rabbit's eye causes corneal opacity and edema.

Reference: 1. Machlowitz, R. A. *et al.* Antibiotics Ann. 806–808, 1954–1955.

Pluramycins

Produced by: Streptomyces pluricolorescens (2).

Method of extraction: Filtered broth at pH 7.0 extracted successively with ethyl acetate. Extract concentrated *in vacuo*. Antibiotics precipitate out on addition of petroleum ether. Also adsorbed on activated carbon or diatomaceous earth at pH 7.0. Purified and separated by chromatographing from an ethyl acetate solution on alumina, or by countercurrent distribution (1 per cent ethyl acetate at pH 6.55 or pH 5.3 phosphate buffer). Pluramycin A is the major component, has the strongest antitumor activity, and can be separated from pluramycin B in the pH 5.3 system. Crystallized from ethanol (2).

Chemical and physical properties: Pluramycin A: Basic. Orange needles (ethanol) or orange prisms (ethyl acetate). Soluble in acidic water, methanol, ethanol, acetone, chloroform, ethyl acetate, butyl acetate, benzene, and ethyl Cellosolve. Slightly soluble in ether and hexane. Insoluble in petroleum ether and water. Needles change color at 154°C, shrink at 157–164°C, and darken at 177°C. Prisms shrink and darken at 200–215°C. Ultraviolet absorption maxima (0.01 mg per ml in ethanol)

at 208 ($E^{1\%}_{1cm}$ 1120) (3) and 245 mμ ($E^{1\%}_{1cm}$ 672) and a shoulder at 265 to 270 mμ ($E^{1\%}_{1cm}$ 430). Infrared spectrum given in reference 2. Prisms: C = 66.63%; H = 6.30%; N = 3.66%. Needles: C = 66.87%; H = 6.61%; N = 3.80%. No S or halogens. Negative $FeCl_3$, Fehling, Tollen, 2,4-dinitrophenylhydrazine, and sodium nitroprusside tests. Purple color in alkaline solution with an absorption maximum at 525 mμ. Yellow in dilute HCl solution with an absorption maximum at 430 mμ. "A"-tartrate is an orange crystalline powder. On paper chromatography (paper pretreated with 0.1 *N* (pH 3.2) tartrate buffer and developed with water-saturated ethyl acetate), Rf = 0.05. Powder stable. Less stable in acid than in neutral or slightly alkaline solution (2). Diffuses slowly in agar (1). *Pluramycin B:* Not completely purified. Reddish brown powder. Soluble in methanol, ethanol, acetone, ethyl acetate, butyl acetate, ether, benzene, chloroform, and ethyl Cellosolve. Insoluble in water and petroleum ether. Differs from A in not being transferred into an aqueous phase from ethyl acetate at pH 5.3. Purple color in alkali. Rf = 0.95 in the system described under A.

Biological activity: Pure pluramycin A inhibits *Staph. aureus* at 0.02 to 0.1 μg per ml (2). Crude powder containing both A and B is also active on other gram-positive bacteria. Very slight to no activity on gram-negative bacteria, yeasts, and filamentous fungi (1). Inhibits growth of both solid and ascitic forms of Ehrlich carcinoma in mice (2).

Toxicity: Pluramycin A: LD_{50} (mice) 25 mg per kg subcutaneously, 6.25 to 12.5 mg per kg intraperitoneally, and 1.25 to 2.5 mg per kg intravenously. Pluramycin A tartrate is more toxic than the free base (4). Causes desquamation and has an antimitotic effect on HeLa cells at 0.06 to 0.15 μg per ml; this activity parallels antitumor activity *in vivo* (2). Contact tests using a crude preparation (A and B) destroy the inoculability of Ehrlich carcinoma cells (1). Active on this tumor in mice. Also active on the solid and ascitic forms of sarcoma 180 (mice). Much less active on Yoshida rat sarcoma (3, 5).

References:

1. Takeuchi, T. *et al.* J. Antibiotics (Japan) **9A:** 22–30, 1956.
2. Maeda, K. *et al.* J. Antibiotics (Japan) **9A:** 75–81, 1956.
3. Umezawa, H. Giorn. microbiol. **2:** 160–193, 1956.
4. Takeuchi, T. *et al.* J. Antibiotics (Japan) **10A:** 143–152, 1957.
5. Nishibori, A. J. Antibiotics (Japan) **10A:** 213–218, 1957.

Primycin

Produced by: Streptomyces sp. (1).

Method of extraction: Acidification of broth-filtrate gives precipitate on which antibiotic is adsorbed. Residue treated with ether, then extracted with methanol. Purification by treatment with colloidal Al $(OH)_3$ and precipitation on addition of ether, or extraction of a 20 per cent methanol solution with butanol and precipitation on addition of ether. Crystallized from methanol-butanol (1).

Chemical and physical properties: White microcrystals; m.p. 166–168°C (decomposition). Soluble in methanol; less soluble in higher alcohols; sparingly soluble in pyridine, glacial acetic acid, and water; insoluble in other solvents. Aqueous solution sensitive to electrolytes. Thermostable. Strongly surface-active. H_2SO_4 solution first yellow then red. Negative Fehling, Elson-Morgan, and maltol tests. No reaction with aldehyde reagents. Positive Brunner-Pettenkoffer and Sakaguchi reactions. Inactivation by acetylation in pyridine reversed by splitting of the acetyl groups. $C_{19}H_{37}O_7N$: C = 58.79%; H = 9.56%; N = 3.63% (Dumas). No unsaturation and probably no guanidino group (1, 2, 4).

Biological activity: Active on gram-positive bacteria, including *Staph. aureus* at 0.02 to 0.06 μg per ml, and mycobacteria. Virucidal. Partly inactivated by serum. Most active at alkaline pH, pH 7.6 to 7.8 being optimal.

Toxicity: LD_{100} (mice) 2.5 mg per kg (no route given). Nontoxic to tissue cultures, and nontoxic to human beings at therapeutic levels (1,2).

Utilization: Topical application (3). Reputedly active on tubercular infections resistant to other antibiotics (2).

References:

1. Vályi-Nagy, T. *et al.* Nature, London **174:** 1105–1106, 1954.
2. Szilágyi, I. and Szabó, I. Arzneimittel-Forsch. **8:** 333–336, 1958.
3. Molnar, G. and Biro, L. Magyar Voor. Lapja **21:** 155–159, 1958.
4. Vályi-Nagy, T. and Kelentey, B. Abstr. Communs. Symposium on Antibiotics, Prague 47–49, 1959.

Proactinomycins

Produced by: Streptomyces (*Proactinomyces*) *gardneri* (1, 2).

Synonyms: Proactinomycin B is similar or

identical to griseomycin, and proactinomycin A is similar or identical to pieromycin (3).

Method of extraction: Broth extracted with amyl acetate at pH 10.0. Extract shaken with acetate buffer at pH 4.0. Aqueous extract adjusted to pH 10.0 and re-extracted into amyl acetate. Amyl acetate distilled *in vacuo*, then extracted three times with water at pH 4. Aqueous extract concentrated by vacuum distillation and lyophilization. Purified by partition between dilute HCl and butyl alcohol-benzene mixtures on a silica gel column. Countercurrent distribution (diethyl ether-0.5 *M* phosphate buffer) indicates the presence of three components. Proactinomycin A is crystallized from a concentrated aqueous ether solution, and recrystallized from chloroform on addition of ether. Proactinomycin B is obtained by addition of light petroleum ether to a concentration of an ether solution of active fractions (3).

Chemical and physical properties: Complex: Basic substance (3). Slightly soluble in water. Soluble in alcohol, acetone, ether, chloroform, and benzene. *Salts:* Soluble in water, alcohol, and acetone. Salts are formed with picric, picrolonic, flavianic, reinecke, and helianthic acids (1). Stable in aqueous solution at pH 2 to 8, but inactivated at room temperature at pH >10.0, and by boiling at pH 2. pK = about 9. Alkaline hydrolysis products include dimethylamine; a base, $C_{18}H_{29}NO_5$; and a neutral substance, $C_6H_9O_2$, from which an osazone can be obtained. *Proactinomycin A:* Short thick plates or short prisms; m.p. 168–169°C. Soluble in chloroform, acetone, alcohol, and dilute acid. Slightly soluble in ether and light petroleum ether. Insoluble in water and dilute alkali. Ultraviolet absorption spectrum maximum at about 240 mμ, with an inflection at about 260 mμ. C = 63.2%; H = 9.04%; N = 2.67%; N—CH_3 = 7.35%; C—CH_3 = 18.8%; O—CH_3 = 1.1%. Molecular weight, 445. $C_{27}H_{47}O_8N$. *Proactinomycin B:* Doubtfully crystalline; m.p. 83–87°C. Ultraviolet absorption spectrum maxima at about 245 and 270 mμ. C = 63.8%; H = 9.4%; N = 2.71%; N—CH_3 = 8.7%; C—CH_3 = 18.04%. Molecular weight, 513. $C_{28}H_{49}O_8N$. *Proactinomycin C:* Not crystallized. Ultraviolet absorption spectrum maxima at about 240 and 295 mμ, with inflections at about 280 and 310 mμ. C = 65.5%; H = 9.2%; N = 3.08%; N—CH_3 = 8.5%; C—CH_3 = 18.6%. Molecular weight, 322. $C_{24}H_{41}O_6N$ (3).

Biological activity: Complex: Active on gram-positive bacteria. Slightly active on *Neisseria meningitidis* and *Sal. enteritidis*, but not on *Sal. typhosa*. Not active on other gram-negative bacteria or mycobacteria (2). Some activity against *Streptococcus pyogenes* infections in mice. Very slightly active (1:10,000) *in vitro* on *Trypanosoma equiperdum* and *Leishmania donovani*. Not active on *Endamoeba histolytica* or fungi (2). Proactinomycins A, B, and C have the same qualitative activity, but B is only one half as active as A and C. A has only slight activity in mice against *Streptococcus pneumoniae* infections (4).

Toxicity: LD_{50} (mice) A: 150 mg per kg, B: 120 mg per kg, C: 80 mg per kg intravenously. *Complex:* 90 mg per kg. Mice tolerate 100 mg per kg orally of A, B, and C, and 100 mg per kg subcutaneously of A (2). About 100 mg per kg of the complex is tolerated intraperitoneally (1). A dilution of 1:3000 kills leucocytes in 1 hour; 1:10,000 is relatively nontoxic (1).

References:

1. Gardner, A. D. and Chain, E. Brit. J. Exptl. Pathol. **23:** 123–127, 1942.
2. Florey, H. W. *et al.* Brit. J. Exptl. Pathol. **26:** 337–349, 1945.
3. Marston, R. Q. Brit. J. Exptl. Pathol. **30:** 398–407, 1949.
4. Marston, R. Q. and Florey, H. W. Brit. J. Exptl. Pathol. **30:** 407–418, 1949.
5. DeSomer, P. Giorn. microbiol. **2:** 216–232 1956.

Prodigiosin-like Antibiotic

Produced by: Streptomyces spp. resembling *S. ruber*.

Method of extraction: Mycelium extracted with benzene in the presence of excess ammonium hydroxide. Concentrated extract gives a precipitate following treatment with gaseous HCl. Purification by chromatography on acid-washed aluminum oxide. Further purification by countercurrent distribution (petroleum ether-benzene-ethanol-0.1 *N* HCl, 4:1:3:2). Crystallized from petroleum ether (1).

Chemical and physical properties: Base: Orange crystals. Partial melting at 147–149°C, followed by recrystallization of the melted portion as the temperature rises; m.p. 203–204°C. Soluble in some organic solvents (1). λ_{max} 294, 331, 471, 529 (90 per cent in ethanol or 90 per cent in ethanol with 0.1 N NaOH). Infrared spectrum given in reference 1. Contains two pyrrole rings, one of which has a β-methoxyl group (2). $C_{25}H_{35}ON_3$: C = 76.78%; H = 8.81%; N = 10.33% (1). *Hydrochloride:* Violet tabular crystals; m.p. 205–209°C. Soluble in most organic solvents, but not in petroleum ether. Insoluble in water. λ_{max} about 295, 357 (broad low peak), 397, and 538 (95 per cent in ethanol) (1, 2).

Biological activity: Moderately active on gram-

positive bacteria. Certain gram-negative bacteria and certain fungi inhibited by 100 μg per ml. Active on *Endamoeba histolytica* at 10 μg per ml (1).

References:

1. Arcamone, F. *et al.* Giorn. microbiol. **4**: 77–88, 1957.
2. Nicolaus, R. A. *et al.* Ricerca sci. **28**: 2314–2317, 1958.

Protocidin

Produced by: Streptomyces sp.

Method of extraction: Mycelium extracted with methanol. Extract concentrated *in vacuo*; protocidin and impurities are precipitated. Extraction with methanol and concentration of extract precipitate the antibiotic. Recrystallized from methanol.

Chemical and physical properties: Tetraene. Ultraviolet absorption spectrum maxima at 277, 290, 303, and 318 mμ. Very soluble in 0.01 *N* HCl or NaOH, glacial acetic acid, or pyridine. Soluble in water, methanol, and benzaldehyde. Slightly soluble in ethanol, butanol, and acetone. Infrared spectrum given in reference 1. Stable to acid and alkali, but not to ultraviolet light. Browns at about 120°C. No clear-cut melting point. Negative biuret, Sakaguchi, Molisch, ninhydrin, anthrone, and $FeCl_3$ tests. Reduces $KMnO_4$. Green reaction with Fehling's solution. Becomes brownish black in concentrated H_2SO_4. Differentiated from other polyenic antibiotics on the basis of paper chromatography (water-saturated butanol), Fehling reaction, infrared spectrum, stability, and solubility.

Biological activity: Not active on bacteria. Active on fungi and yeasts. Inhibits *Trichomonas vaginalis* at 50 μg per ml.

Toxicity: Mice tolerate 30 mg per kg intravenously.

Reference: 1. Sakamoto, J.M.J. J. Antibiotics (Japan) **10A**: 128–131, 1957.

Protomycin

Produced by: Streptomyces sp., strain S-300.

Chemical and physical properties: Stable, oily substance. Most soluble in 5 per cent aqueous acetone.

Biological activity: No activity against bacteria. Active against *Endamoeba histolytica in vitro* and *in vivo*.

Toxicity: No toxic effect if 50 mg per kg administered orally to guinea pigs.

Reference: 1. Hirabayashi, A. J. Antibiotics (Japan) **12A**: 298–309, 1959.

Pseudostreptomycin

Produced by: Streptomyces griseus.

Method of extraction: Extracted from broths along with streptomycin. Separated from the latter by fractional precipitation from a 90 per cent methanol-10 per cent water solution of streptomycin trihydrochloride on addition of 10 per cent of the quantity of triethylamine sulfate in methanol calculated to completely precipitate the streptomycin as the sulfate. Converted to the trihydrochloride, washed with methanol, and crystallized as the reineckate from water. Can be converted to streptomycin in the presence of acid at pH 2.5 at 50°C.

Chemical and physical properties: Colorless substance. Condensation product of two streptomycin molecules linked at their carbonyl groups by an amine bridge. Cannot be hydrogenated catalytically under conditions which give a dihydro derivative of streptomycin.

Biological activity: Has about one tenth the activity of streptomycin by weight.

Toxicity: LD_0 (mice) 12.5 μg per kg intravenously (sulfate).

Reference: 1. Solomons, I. A., III U. S. Patent 2,565,402, August 21, 1951.

Psicofuranine

Produced by: Streptomyces hygroscopicus var. *decoyicus.*

Synonym: Closely related to angustmycin C.

Method of extraction: Culture adjusted to pH 2.0 and filtered. Filtrate adjusted to pH 9.7 to 10.0 and adsorbed on Nuchar C-190 (1 per cent). Elution with 80 per cent acetone at pH 9.7 to 10.0. The eluate is adjusted to pH 7.0 to 8.0 and concentrated to an aqueous solution. Upon standing at 2°C, crystallization takes place. Further purification by countercurrent distribution in *n*-butanol and water. Psicofuranine has a *K* value of 0.28 to 0.35 in that system.

Chemical and physical properties: White crystals; m.p. 212–214°C (decomposition). $C_{11}H_{15}N_2O_5$. Very soluble in dimethylformamide, dimethyl sulfoxide, and hot water. Soluble as follows at room temperature: water and methanol, 8 mg per ml; ethanol, 6 mg per ml; n-butanol, 2 mg per ml; ethyl acetate, 0.23 mg per ml. $[\alpha]_D^{25} = -53.7°$ (c = 1 per cent in dimethyl sulfoxide) and −68° (c = 1 per cent in dimethylformamide). Half-life 18 hours at pH 2.0 and 30°C; more stable at neutral and alkaline pH. Maximal light absorption at 259 mμ ($E_{1\,cm}^{1\%}$ 508) in 0.01 *N* acid, and at 261 mμ ($E_{1\,cm}^{1\%}$ 527) in 0.01 *N* base. Infrared absorption

spectrum given in reference 1. Paper chromatography and ultraviolet light-absorption assay given in reference 5.

Biological activity: Activity *in vitro* difficult to demonstrate. Active against some gram-positive and gram-negative bacteria when a special semisynthetic medium is used: KH_2PO_4, 3.0 gm; Na_2HPO_4, 3.0 gm; $MgSO_4 \cdot 7H_2O$, 0.11 gm; NH_4Cl, 1.0 gm; deionized water, 1 liter; liver extract (3, 4). Active in mice, orally and subcutaneously, against infections caused by *Staph. aureus*, *Streptococcus hemolyticus*, and *E. coli* (2). Active orally in rats against Walker adenocarcinoma, Murphy-Sturm lymphosarcoma, Jensen sarcoma, and Guérin tumor. No activity in mice with either the ascitic or solid forms of Ehrlich carcinoma, sarcoma 180, leukemia L-1210, or spontaneous mammary adenocarcinoma (6). Psicofuranine can be determined by a chemical method in plasma and serum (7).

References:

1. Eble, T. E. *et al.* Antibiotics & Chemotherapy **9:** 419–420, 1959.
2. Lewis, C. *et al.* Antibiotics & Chemotherapy **9:** 421–426, 1959.
3. Vavra, J. J. *et al.* Antibiotics & Chemotherapy **9:** 427–431, 1959.
4. Hanka, L. G. *et al.* Antibiotics & Chemotherapy **9:** 432–435, 1959.
5. Sokolski, W. T. *et al.* Antibiotics & Chemotherapy **9:** 436–438, 1959.
6. Evans, J. S. and Gray, J. E. Antibiotics & Chemotherapy **9:** 675–684, 1959.
7. Forist, A. A. *et al.* Antibiotics & Chemotherapy **9:** 685–689, 1959.

Pulvomycin

Produced by: Streptomyces sp.

Synonym: Closely related to mycolutein; also produced by same culture.

Method of extraction: Mycelium, containing most of the active substance, extracted with methanol. Extract concentrated *in vacuo* below 50°C, filtered, and extracted with chloroform. Addition of petroleum ether to the chloroform concentrate precipitates pulvomycin. Chromatographed on alumina from a methanolic solution and eluted with methanol. Active fractions concentrated to dryness *in vacuo*. Residue extracted with chloroform and precipitated with petroleum ether.

Chemical and physical properties: Amorphous powder; m.p. 94–97°C. Soluble in chloroform, acetone, lower alcohols, benzene, dioxane, pyridine, glacial acetic acid, and ethyl acetate. Insoluble in carbon tetrachloride, ethyl ether, petroleum ether, water, dilute HCl, Na_2CO_3 solution, and 0.5 *N* NaOH. Black color with concentrated H_2SO_4. Positive test for aromatic nucleus ($AlCl_3$ and $CHCl_3$). Negative ninhydrin, Sakaguchi, maltol, creatinine, $FeCl_3$, Elson-Morgan, Molisch, biuret, and Br_2 in CCl_4 tests. C = 65.69%; H = 7.45%; N = 1.85% (Kjeldahl). Rf = 0.94 on paper chromatography using water-saturated butanol containing 2 per cent toluenesulfonic acid. Ultraviolet absorption spectrum has maxima at 275 and 316 mμ. Infrared data given in reference 1. $[\alpha]_D^{25} = -82°$ (c = 1 per cent in $CHCl_3$) and $-183°$ (c = 1 per cent in methanol).

Biological activity: Active on gram-positive bacteria, including mycobacteria. Not active *in vivo*.

Toxicity: Mice tolerate 250 mg per kg intraperitoneally.

Reference: 1. Zief, M. *et al.* Antibiotics & Chemotherapy **7:** 384–386, 1957.

Puromycin

Produced by: Streptomyces alboniger (10).

Synonyms: Achromycin (this now is a trade name for tetracycline); stylomycin.

Chemical and physical properties: Diacidic base. White crystals; m.p. 175.5–177°C. Sparingly soluble in water and organic solvents (33). $[\alpha]_D^{25} = -11°$ (ethanol). Ultraviolet absorption spectrum maximum at 275 mμ ($E_{1\,cm}^{1\%}$ 203) (0.1 *N* NaOH) or 267.5 mμ ($E_{1\,cm}^{1\%}$ 195) (0.1 *N* HCl). Stable pK_a = 6.8 and 7.2. *Dihydrochloride:* m.p. 174°C (decomposition). *Sulfate:* White crystals; m.p. 180–187°C (decomposition). *Picrate:* m.p. 217.5–218°C. On acid hydrolysis, the ultraviolet absorption maxima are shifted 5 to 10 mμ to the longer wave length and biological activity is destroyed (1, 3, 32). Infrared spectrum given in reference 1. The biologically inactive alcoholysis products include: the amphoteric, water-soluble, acidic 6-dimethyladenine ($C_7H_9N_5$), m.p. 257–258°C; the ester of *p*-methoxy-L-phenylalanine; and 3-amino-D-ribose (9, 24). C = 56.55%; H = 6.35%; N = 20.88%. $C_{22}H_{29}N_7O_5$ (1, 3, 12, 24). Puromycin is 6-dimethylamino-9-(3′-*p*-methoxy-L-phenylalanylamino-3′-desoxy-β,D-ribofuranosyl)purine (4, 12, 26). Structural formula given in Chapter 6.

Biological activity: Active on gram-positive bacteria; less active on gram-negative bacteria (1). Active on protozoa (2, 18). Active on *Chlorella pyrenoidosa* at 10^{-2} *M* (31). Some protective or curative effect on *Trypanosoma* infections of mice and rabbits (2, 8, 20). Prolongs survival time of mice infected with *Toxoplasma gondii* (6). Protects against *Endamoeba histolytica* infections in rats

and guinea pigs (14). Curative action on *Babesia rodhani* infections of mice (30). Active on natural oxyurid and tapeworm infections in mice (27). Slightly active, in contact tests, on herpes simplex virus infections of mice (23). Some inhibition of the following neoplastic diseases: mammary adenocarcinoma (C3H mice), 755 mammary adenocarcinoma (C57BL mice), transplanted leukemia (mice), Lewis bladder carcinoma, Ridgeway osteogenic sarcoma, Harding-Passey melanoma, 72J mammary carcinoma, 8110 brain carcinoma, carcinoma 1025, Bashford carcinoma 63, and Miyono adenocarcinoma (5, 11, 12, 21, 24, 32). The carcinostatic activity parallels the trypanocidal activity (18), but not the antibacterial activity (24). The antitumor activity of puromycin was shown to reside in the aminonucleoside portion of the molecule (17). (See also "Puromycin Analogues" section which follows.)

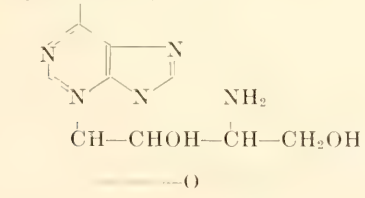
Toxicity: LD_{50} (mice) 335 mg per kg intravenously, 580 mg per kg intraperitoneally, and 720 mg per kg orally. Not irritating to rabbit's eye or by parenteral administration (16).

Utilization: Amoebic dysentery (28), chronic amebiasis (13), sleeping sickness (29). Temporary effect on some advanced incurable disseminated neoplastic diseases in human beings (22).

Puromycin Analogues

Produced by: Synthesis from puromycin (12).

Chemical and physical properties: Aminonucleoside analogue 2484L: Crystalline substance containing the amino sugar and purine moieties of puromycin; m.p. 215–216°C. $[\alpha]_D^{25} = -24.6°$ (H_2O). C = 49.4%; H = 6.39%; N = 28.4%. It is 6-dimethylamino-9-(3′-amino-3-desoxy-β,D-ribopuranosyl)purine. Structural formula (12):



Other analogues: These include analogues in which the amino acid moiety has been replaced by a different amino acid or a peptide (17).

Biological activity: Analogue 2484L: Does not have the antibacterial spectrum typical of puromycin; 3 to 4 times more active against *Trypanosoma equiperdum* (17). On a molecular basis, it is equally as active as puromycin against *T. equiperdum* (7). It has considerably less activity against *Endamoeba histolytica* infections (guinea pigs) than puromycin (15). This analogue is very active (more so than the parent compound) against transplanted mammary adenocarcinoma in C3H mice and 755 mammary adenocarcinoma in C57BL mice (12, 17, 24). *Other analogues:* Four amino acid analogues, L-phenylalanyl, glycyl, leucyl, and glycyl-*p*-methoxy-L-phenylalanyl, have an inhibitory effect on transplanted mammary adenocarcinoma of mice and are more active than puromycin. The L-phenylalanyl derivative also has some antileukemic activity (17). Six other analogues, carboethoxyamino, benzylideneamino, α-sodium sulfobenzylamino, L-phenylalanylamino, 4-chlorobenzylideneamino, and m-methoxy *p*-hydroxybenzylideneamino substitutions for the amino acid moiety of puromycin, have activity equal to the 2484L analogue against *Trypanosoma equiperdum* (19). Fifteen benzylidene analogues of puromycin were active on mammary adenocarcinomas in mice (33).

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Pyridomycin

Produced by: Streptomyces pyridomyceticus (6, 7), formerly *S. albidofuscus* (1, 2); and *Streptomyces* sp. (3).

Method of extraction: I. Broth extracted with butyl acetate. Extract concentrated to a syrup *in vacuo*. Addition of ether or petroleum ether and cooling gives crystals. Taken up in chloroform, concentrated, and recrystallized from ethanol. Can also be adsorbed on activated carbon and eluted with ethanol or acetone, or extracted from mycelium with acetone (1, 5). II. Broth-filtrate at pH 7.4 passed through a column of Ionex C cation exchange resin (H^+ form). Column washed with water and 0.5 *N* HCl and eluted with 80 per cent acetone. Active fractions adjusted to pH 7.4 and evaporated *in vacuo*. Residue extracted with ethyl acetate. Extract concentrated and chromatographed on aluminum oxide. Column washed with ethyl acetate, methanol, and acetone, and eluted with 80 per cent aqueous acetone. Evaporation of the effluent gives crystals. Recrystallized from acetone-water (3).

Chemical and physical properties: Colorless needles; m.p. 221–222°C (5) or 214–216°C (3). Very soluble in chloroform. Soluble in lower alcohols, ethyl or butyl acetate, benzene, ethyl Cellosolve, acetone, dioxane, and tetrahydrofuran. Slightly soluble in ether and petroleum ether. Water solubility increased by addition of HCl; also increased by alkali, but with a concomitant loss of biological activity. Ultraviolet absorption spectrum maxima at 303 mμ ($E^{1\%}_{1cm}$ 209) (alcohol); or 227 mμ ($E^{1\%}_{1cm}$ 505) and 303 mμ ($E^{1\%}_{1cm}$ 250) (0.1 *N* HCl); or 331 mμ ($E^{1\%}_{1cm}$ 166) (0.1 *N* NaOH) (1, 5). Infrared spectrum given in reference 5. Positive $FeCl_3$, biuret, and Rosenthaler tests. Negative ninhydrin, Benedict, Fehling, and Sakaguchi tests. Stable at an acid reaction but not at an alkaline. No methoxyl or acetyl groups; cannot be hydrogenated. Contains a pyridine nucleus. $C_{26\text{-}27}H_{32}N_4O_8$: C = 60.62%; H = 5.95%; N = 10.37%. *Hydrochloride:* White needles; m.p. 194–196°C (decomposition). Freely soluble in water. Ultraviolet absorption spectrum maxima at 226 ($E^{1\%}_{1cm}$ 669) and 303 mμ ($E^{1\%}_{1cm}$ 332). Infrared absorption spectrum given in reference 5. $[\alpha]^{19}_{D}$ = −53.2° (c = 1.0 per cent in water). Alkali fusion products include β-picoline, 2-ethyl malic acid, 3-hydroxypicolinic acid, glycine, and leucine or isoleucine. Mild acid hydrolysis products include a substance containing a pyridine nucleus, $C_{20}H_{22}N_4O_9$. Strong acid hydrolysis products include L-threonine (5).

Biological activity: Active against mycobacteria. Active on *K. pneumoniae* (3 μg per ml), *Sal. paratyphi* A (6 μg per ml), and *E. coli* (25 μg per ml), but not on other bacteria tested (1, 5). Slightly inhibits ascites increase in mice with Ehrlich carcinoma (4).

Toxicity: Base: Mice tolerate 1998 mg per kg intraperitoneally. *Hydrochloride:* LD_{50} (mice) = 300 mg per kg intraperitoneally (5).

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Questiomycins

Produced by: Streptomyces sp. which also produces a eurocidin-like antibiotic.

Synonyms: Questiomycin A is 6-aminophenoxazone; questiomycin B is o-aminophenol. These two chemicals were known to have antitubercular activity before the discovery of questiomycins, but were not known to be produced by actinomycetes. 6-Aminophenoxazone is closely related to the chromophore of actinomycin.

Method of extraction: Broth-filtrate extracted with ethyl acetate. The solvent layer extracted with water at pH 2.5. The water layer contains questiomycin B, and the ethyl acetate contains questiomycin A. *Questiomycin A:* Ethyl acetate layer extracted with *N* HCl. The pH of the aqueous layer is brought up to 2.5; extracted with benzene and concentrated to yield crude questiomycin A. The crude questiomycin A is dissolved in ethyl acetate and passed through a column of alumina. Elution is carried out with ethyl acetate until a red pigment is completely eluted; the eluate is concentrated. Upon standing, a precipitate is formed which is collected, dissolved in anhydrous benzene, and chromatographed through a column of magnesium silicate. A red material is eluted with a benzene-ethyl acetate mixture (4:1). The eluate is concentrated, bringing about crystallization. Further purification by sublimation *in vacuo* at 150°C and recrystallization from ethyl alcohol. *Questiomycin B:* The water layer is adjusted to pH 6.0 and extracted with ethyl acetate; evaporated to yield crude questiomycin B. The crude questiomycin B is dissolved in anhydrous ethyl acetate, and chromatographed over a column of alumina. The column is washed with ethyl acetate, acetone, and methyl alcohol. Elution with water. Water extracted with ethyl acetate; evaporated to dryness. Further purification by sublimation *in vacuo* at 120°C and recrystallization from benzene.

Chemical and physical properties: Questiomycin A: Red crystals. Sublimes above 150°C; m.p. 241–244°C (decomposition). C = 67.31%; H = 3.98%; N = 12.96%. $C_{12}H_8O_2N_2$. Molecular weight, 212.2 (or Rast, 217). Soluble in dimethylformamide, glacial acetic acid, and concentrated hydrochloric acid. Slightly soluble in ether, benzene, ethyl acetate, chloroform, acetone, ethyl alcohol, and methyl alcohol. Insoluble in petroleum ether and water. Light-absorption maxima at about 465 and 230 mμ in 0.1 *N* HCl; 330 mμ with end-absorption in 0.1 *N* NaOH; 425, 410, and 240 mμ in cyclohexane. Unstable at alkaline reaction. Infrared light absorption given in reference 1. Questiomycin A was found to be identical with 6-aminophenoxazone. (Structural formula given in Chapter 6.) *Questiomycin B:* Colorless crystals, browning when exposed to atmosphere. Sublimes at 120°C; m.p. 170–175°C. C = 65.80%; H = 6.23%; N = 12.68%. C_6H_7NO. Molecular weight, 109.12 (found, 103). Soluble in water, methyl alcohol, ethyl acetate, and ether. Sparingly soluble in carbon tetrachloride and petroleum ether. Light-absorption maxima at about 230 and 285 mμ with end-absorption in water. Infrared absorption spectrum given in reference 1. Questiomycin B was found to be identical with o-aminophenol. (Structural formula given in Chapter 6.) This compound is believed to be the building stone of 6-aminophenoxazone.

Biological activity: Active mainly against mycobacteria. Some activity against *B. subtilis*, *Monilia formosa*, *Schizosaccharomyces astospora*, and *P. chrysogenum*.

Toxicity: LD_{50} (mice) questiomycin A: >400 mg per kg, questiomycin B: 300 mg per kg intraperitoneally.

Reference: 1. Anzai, K. *et al.* J. Antibiotics (Japan) **13A:** 125–132, 1960.

Quinocyclines

Produced by: Streptomyces aureofaciens (1).

Method of extraction: Quinocycline complex: Broth-filtrate from 2½-day-old fermentation extracted with ethyl acetate. Extract concentrated. Addition of hexane precipitates the complex. *Quinocycline A and isoquinocycline A:* Broth-filtrate from 1-day-old fermentation extracted with methyl isobutyl ketone. Extract treated with glacial acetic acid and concentrated *in vacuo*. Concentrate extracted with 0.2 per cent aqueous acetic acid. Aqueous phase adjusted to pH 7 to 7.5 and extracted with methylene chloride. Extract treated with glacial acetic acid and CCl_4. Evaporation of methylene chloride from mixture *in vacuo*, and CCl_4 remaining extracted with distilled water. Water-extract freeze dried. Reprecipitated from methylene chloride to give quinocycline A acetate. "Second crop" crystals from filtrates of methylene chloride give mixture (I) of quinocycline A and its isomer, isoquinocycline A. Purification of I: Methanolic solution stirred with addition of acetone to precipitate isoquinocycline A. *Quinocycline B and isoquinocycline B:* Broth-filtrate from a 3-day-old fermentation extracted with methyl isobutyl ketone. Extract stirred with 0.2 per cent aqueous acetic acid. Aqueous phase collected and adjusted to pH 7.5, while stirring with methylene chloride. Solvent treated with

glacial acetic acid and evaporated *in vacuo* to remove all methylene chloride. Residual solution extracted with distilled water. Water treated with pH 4.1 acetate buffer and extracted with methylene chloride. Extract evaporated to dryness *in vacuo*. Triturated in ether. Separation of components quinocycline A and B and isoquinocycline B by countercurrent distribution (acetate buffer, pH 4.1, and methylene chloride-CCl_4, 1:1). Quinocycline B acetate is treated with HCl in ether, and chilled by evaporation to give quinocycline B-HCl (1).

Chemical and physical properties: Complex: Brilliant yellow solid. Contains four biologically active components which show the following Rf values on paper chromatography (chloroform-ethyl acetate-formamide): 0.15 (quinocycline A), 0.25 (isoquinocycline A), 0.35 (quinocycline B), 0.40 (isoquinocycline B). The complex and all components have ultraviolet absorption spectrum maxima at 228, 258, and 425 mμ. Methanolic 0.01 *N* HCl solutions are bright yellow; methanolic 0.01 *N* NaOH solutions are red. Concentrated H_2SO_4 solutions are deep red, turning purple on standing. *Quinocycline A:* $[\alpha]_{Hg}^{25} = +159°$ (methanol). Aqueous solution at pH 7.0 is red, stays red on dilution. HCl salt: C = 60.46%; H = 5.8%; N = 4.13%; Cl = 5.37%. *Quinocycline B:* $[\alpha]_{Hg}^{25} = +140°$ (methanol). Aqueous solution at pH 7.0 is red, turns orange on dilution. C = 60.5%; H = 5.8%; N = 3.9%; Cl = 4.9%. *Isoquinocycline A:* $[\alpha]_{Hg}^{25} = +37°$ (methanol). Aqueous solution at pH 7.0 forms yellow precipitate, soluble on dilution. C = 60.81%; H = 5.86%; N = 3.86%; Cl = 4.84%. *Isoquinocycline B:* $[\alpha]_{Hg}^{25} = +24°$ (methanol). Aqueous solution at pH 7.0 same as A. C = 60.71%; H = 5.72%; N = 3.71%; Cl = 4.84%. *Degradation products:* Acid degradations of quinocyclines A and B give the same colored product, *quinocycline*, and dissimilar colorless substances, *compound A* and *compound B*, respectively. On acid degradation, isoquinocyclines A and B give a common product, *isoquinocycline* (the isomer of the acid degradation product, quinocycline) and the same two dissimilar products, compound A and compound B. *Quinocycline:* HCl: Bright orange needles. $[\alpha]_{Hg}^{25} = +244°$ (methanol). Aqueous solution adjusted to pH 7.0 is a red gel, unchanged by dilution. Rf = 0.10 (same system given under "Complex"). Amphoteric substance. Same ultraviolet absorption spectrum as complex. C = 62.15%; H = 4.37%; N = 5.74%; Cl = 7.01%. *Isoquinocycline:* HCl: Reddish orange prisms. $[\alpha]_{Hg}^{25} = +29°$ (methanol). Aqueous solution adjusted to pH 7.0 gives an orange-yellow precipitate, partially dissolved by dilution. Rf = 0.20 (same system as above). Amphoteric substance. Same ultraviolet absorption spectrum as complex. C = 62.10%; H = 4.29%; N = 5.61%; Cl = 6.98%. *Compound A:* Colorless neutral crystals; m.p. 151–152°C. No characteristic ultraviolet absorption spectrum. Negative $FeCl_3$, Tollen, Fehling, and 2,4-dinitrophenylhydrazine tests. $C_8H_{14}O_4$. Highly water-soluble. $[\alpha]_D = -111°$ (water). *Compound B:* Colorless neutral oil. Positive Fehling, Tollen, and 2,4-dinitrophenylhydrazine tests. Negative $FeCl_3$ test. Melting point of the hydrazine, 99–100°C. No characteristic ultraviolet absorption spectrum (1).

Biological activity: Complex: Active on gram-positive bacteria (0.78 to 12.5 μg per ml), including mycobacteria (0.019 to 1.56 μg per ml). Not active on gram-negative bacteria or *C. albicans*. Active in only a small range on tuberculosis in mice. *Quinocycline A and B:* Comparable to complex in activity on gram-positive organisms. Up to 10 times more active on mycobacteria. Quinocycline B is the more active. Active in a small range on tuberculosis in mice. *Quinocycline* (degradation product): Comparable to complex. Active in a small range on tuberculosis in mice. *Isoquinocycline* (degradation product): Less active on gram-positive bacteria and nonpathogenic mycobacteria, but comparable to complex against pathogenic mycobacteria (2). *Isoquinocyclines A and B:* Reputedly less active than their isomers (1).

Toxicity: Complex: Mice tolerate 25 mg per kg subcutaneously, with no toxic reactions, and 200 mg per kg orally. *Quinocycline:* Mice tolerate 12.5 mg per kg subcutaneously, with no toxic reactions, and 50 mg per kg orally. *Quinocycline A and B:* A is tolerated by mice at 6.25 mg per kg subcutaneously, and 200 mg per kg orally. B is more toxic (2).

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Racemomycins

Produced by: Streptomyces racemochromogenus (3).

Synonym: The original antibiotic 229 may resemble streptothricin III. Racemomycin B belongs to the streptothricin-geomycin group.

Remarks: This culture was originally (1, 2) reported to produce a basic, water-soluble substance termed 229. Later (3) it was found that another substance, 229B, was being formed instead of 229.

Mutants of *S. racemochromogenus* were then isolated (4), and three substances, racemomycins A, B, and C, were described. Racemomycin B is synonymous with substance 229 B. Racemomycin B has been most thoroughly investigated. The relationship of the original 229 substance to racemomycin A or C is not known.

Method of extraction: Essentially like that of streptomycin or streptothricin (3, 4).

Chemical and physical properties: Original 229: Basic substance. Colorless. Soluble in water. *Hydrochloride:* m.p. 138–140°C. *Sulfate:* m.p. 198–200°C (decomposition). *Reineckate:* m.p. 169–171°C (decomposition). *Helianthate:* m.p. 234–239°C (decomposition). Positive Molisch and Elson-Morgan tests. Negative Sakaguchi, maltol, Fehling, $FeCl_3$, biuret, and ninhydrin tests. Most stable to boiling at pH 5.0; moderately stable at pH 2.0; least stable at pH 9.0. *Racemomycin B* (229B): Basic substance, containing strong and weak basic groups. *Free Base:* White hygroscopic powder; m.p. 150°C (decomposition). Infrared spectrum given in reference 4. $[\alpha]_D^{19} = -34°$ (c = 0.5 per cent in water). Positive Molisch, Elson-Morgan, biuret, and ninhydrin tests. Negative Sakaguchi, maltol, $FeCl_3$, 2,4-dinitrophenylhydrazine, and Fehling tests. Gives a white precipitate with both $HgCl_2$ and phosphotungstic acid (4). Data on paper chromatography given in references 3 and 4. $C_{60}H_{128}O_{32}N_{20}$: C = 41.53%; H = 8.29%; N = 16.51% (4, 6). Hydrolysis products include β-lysine, streptolidine (geamine, roseonine) (5), and a reducing sugar (4). Racemomycin B is considered to differ from geomycin and roseothricin because the ratios of the amounts formed of these products from one mole of each antibiotic differ (6). *Hydrochloride:* White hygroscopic powder; m.p. 175°C (decomposition). $[\alpha]_D^{19} = -45°$ (c = 0.5 per cent in water). *Sulfate:* White hygroscopic powder; m.p. 203°C (decomposition). *Helianthate:* Dark red, amorphous powder; m.p. 210°C (decomposition). *p-(p-Hydroxyphenylazo)benzene sulfonate:* Orange-yellow amorphous substance; m.p. 207°C. *Picrate:* Yellow hygroscopic powder; m.p. 198°C. Titration data given in reference 4. *Reineckate:* Purple (4); m.p. 172–175°C (decomposition) (3). *N-Benzoyl derivative:* m.p. 209°C (decomposition) (4). *Racemomycin C:* Closely related to racemomycin B. Isolated as the *p*-(*p*-hydroxyphenylazo)benzene sulfonate salt; m.p. 210°C (decomposition). $C_{15}H_{32}O_8N_5$ (4).

Biological activity: Original 229: Active on gram-positive and gram-negative bacteria. Cross-resistance with streptothricin but not streptomycin. Diffuses faster in agar than 229B or streptomycin (3). *Racemomycin B:* Activity resembles 229, but is less active on mycobacteria (3).

Toxicity: Original 229 (crude): LD_{50} (mice) 216 μg per kg (no route given). No delayed toxicity (3). *Racemomycin B* (crude). LD_{50} (mice) 62.3 mg per kg (intravenously). Has considerable delayed toxicity.

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Ractinomycins

Produced by: Streptomyces sp. closely related to *S. phaeochromogenes* (1, 3).

Method of extraction: I. Broth-filtrate extracted with butyl acetate. Extract concentrated *in vacuo* and chromatographed on alumina. Developed with ethyl acetate. Eluate concentrated and cooled, giving crude ractinomycin. Crystals extracted with ether; ether concentrated and cooled to give *ractinomycin A*. Ether-insoluble residue taken up in ethyl acetate, then cooled to give ractinomycin B (1). II. Broth adjusted to pH 5.0 and filtered. Antibiotic adsorbed on active clay, and eluted with acetone. Antibiotic precipitates when the extract is concentrated *in vacuo*. Precipitate and liquor extracted with ethyl acetate. Extract concentrated *in vacuo* and cooled to give orange crystals of ractinomycin A. Recrystallization from ether. Mycelium extracted with acetone (3).

Chemical and physical properties: Ractinomycin A: Orange needles. Turns brown at 157–158°C and blackens at 205°C. Most soluble in chloroform and acetone; soluble in ethyl and butyl acetates; slightly soluble in methanol, ethanol, benzene, and carbon disulfide; almost insoluble in petroleum ether and water. λ_{max}^{EtOH} 245 mμ ($E_{1cm}^{1\%}$ 780), 440 to 450 mμ ($E_{1cm}^{1\%}$ 220). Infrared spectrum given in reference 3. Positive Tollen, $FeCl_3$, and Molisch tests. Decolorizes $KMnO_4$. A sodium carbonate solution of A is decolorized by H_2O_2. In aqueous solution, turns from yellow to purple at pH 6.4 to 6.6; above this pH it is not stable. In concentrated H_2SO_4, turns from reddish violet to blue; in con-

centrated HCl, orange color changes to violet on heating. Negative ninhydrin, biuret, Sakaguchi, and Millon tests. Rf values on paper chromatography given in reference 3. Stable at room temperature between pH 2.0 and 10.0. Destroyed by heating at 100°C for 20 minutes at pH above 7.0, but stable between pH 2.0 and 6.0 (1). $C_{33}H_{30}$–N_3O_{14} : C = 57.3%; H = 4.25%; N = 6.07%. No S or halogens. Acid hydrolysate contains no ninhydrin-positive substances. *Ractinomycin B:* Reddish orange needle-shaped crystals; m.p. 172–175°C (decomposition). Same solubility properties as A, but, as a rule, less soluble. Insoluble in concentrated HCl and 10 per cent NaOH. λ_{max}^{EtOH} 250 ($E_{1cm}^{1\%}$ 1080) and 400 to 450 ($E_{1cm}^{1\%}$ 260). $FeCl_3$ test negative. Other tests and stability as for ractinomycin A. Contains no halogen or S (1, 3).

Biological activity: Ractinomycins A and B are active *in vitro* against gram-positive bacteria and fungi. A is more active than B against gram-positive bacteria but less active against mycobacteria and fungi. B also has a small amount of activity (50 to 100 μg per ml) against gram-negative bacteria. Both ractinomycins are active against *Toxoplasma gondii* (1). A has antitumor activity against the ascitic form of Ehrlich carcinoma, and causes the disappearance of HeLa cells in mitosis at 25 mg per ml (2, 3).

Toxicity: Ractinomycin A: LD_{50} (mice) 10 mg per kg intraperitoneally (1), 5 to 12.5 mg per kg intravenously (3). *Ractinomycin B:* Mice tolerate 25 mg per kg intraperitoneally (1).

References:

1. Utahara, R. *et al.* J. Antibiotics (Japan) **8A:** 132–135, 1955.
2. Umezawa, H. Giorn. microbiol. **2:** 160–193, 1956.
3. Utahara, R. J. Antibiotics (Japan) **10A:** 115–119, 1957.

Raisnomycin

Produced by: Streptomyces kentuckensis (1).

Method of extraction: Broth adjusted to pH 2.5 and filtered. Filtrate adjusted to pH 9 and extracted with n-butanol. Butanol evaporated to dryness. Residue taken up in 0.1 *N* HCl and evaporated to dryness.

Chemical and physical properties: Dark yellow basic substance. Insoluble in water, acetone, and ether. Soluble in ethanol, methanol, butanol, and isopropanol. Hydrochloride and sulfate slightly soluble in water. No end-absorption in ultraviolet light.

Biological activity: Moderately active on gram-positive and gram-negative bacteria, but not on *K. pneumoniae, Ps. aeruginosa,* mycobacteria, or yeasts.

Toxicity: LD_{50} (rats) 28 to 35 mg per kg (route not given).

Reference: 1. Barr, F. S. and Carman, P. E. Antibiotics & Chemotherapy **6:** 286–289, 1956.

Ramnacin

Produced by: Streptomyces ramnaii (2).

Method of extraction: Extraction of culture-filtrate with ether at pH 8.0. Extraction of the mycelium with ethanol; evaporation of ethanol to dryness. Residue suspended in water at pH 8.0; extraction with ether. The two ether-extracts combined and evaporated to dryness. The residue suspended in chloroform and chromatographed on activated alumina. The active material moves almost with the solvent front. Crystallization from a petroleum ether-alcohol mixture.

Chemical and physical properties: Stable substance (can be autoclaved at 15 pounds per square inch for 20 minutes at pH 2 to 10); m.p. 235°C. Tentative empirical formula: $C_{26}H_{43}O_8$. Sparingly soluble in water. Very soluble in alcohol, acetic acid, ethyl acetate, chloroform, and ether. Moderately soluble in benzene and petroleum ether. Does not decolorize bromine in carbon tetrachloride. Positive test for benzenoid structure. Paper chromatography (n-butanol-acetic acid-water, 4:1:5) and countercurrent distribution of 19 transfers (petroleum ether, b.p. 80–90°C, 95 per cent ethanol and water, 4:3:1) reveal only one active component. (1).

Biological activity: Active *in vitro* against gram-positive bacteria and one strain of *Pr. vulgaris.* No activity against one strain of *Ps. aeruginosa.* Active against two fungi out of nine tested.

References:

1. Ahmad, K. and Islam, M. F. Nature, London **176:** 646–647, 1955.
2. Ahmad, K. *et al.* Ann. Biochem. and Exptl. Med. (Calcutta) **15:** 175–180, 1955.

Raromycin

Produced by: Streptomyces albochromogenes, closely related to *S. griseochromogenes* (blasticidin-producer) (3).

Method of extraction: Mycelium extracted with 60 to 80 per cent acetone. Extract concentrated and extracted with butanol. Broth-filtrate extracted with butanol. Active substance precipitated on addition of ether (2).

Chemical and physical properties: m.p. 211–213°C. Soluble in alkaline water, methanol, ethanol, aqueous butanol, aqueous acetone, diox-

ane, and pyridine. Slightly soluble in acetone butanol, and isopropyl alcohol. Insoluble in acidic water, benzene, ether, petroleum ether, hexane, carbon tetrachloride, ethyl and butyl acetate. No characteristic maxima in ultraviolet light-absorption spectrum. Infrared spectrum indicates a carboxyl group. Titration and solubility indicates the presence of an acidic group. Contains acetylable hydroxyl groups. $[\alpha]_D^{25} = +22°$, C = 57.97%; H = 8.46%; N = 0.44% (3).

Biological activity: Antitumor activity on ascites and solid forms of Ehrlich carcinoma and Crocker sarcoma 180 in mice and Yoshida sarcoma (ascitic) in rats. Retards mitosis of HeLa cells at 15 to 125 μg per ml and causes degeneration at 250 μg per ml (2). Not active on microorganisms (3).

Toxicity: Mice tolerate 200 mg per kg intravenously, and 1 gm per kg intraperitoneally (1).

References:

1. Tanaka, N. *et al.* J. Antibiotics (Japan) **10A:** 189–194, 1957.
2. Tanaka, N. *et al.* Gann **48:** 445–447, 1957.
3. Tanaka, N. *et al.* J. Gen. Appl. Microbiol. **4:** 259–271, 1958.

Resistomycin

Produced by: Strains of *Streptomyces resistomycificus* (2) and variants thereof (3), and *S. arabicus* (4).

Synonym: Croceomycin (4).

Method of extraction: Dried powdered mycelium treated with petroleum ether, then extracted with ether. Antibiotic crystallized from boiling ether, and from ether concentrate. Chromatographed on calcium sulfate from dioxane-ether (1:10). Crystallized from acetone or dioxane (3).

Chemical and physical properties: Weakly acidic substance. Yellow needles; m.p. 315°C (decomposition). Becomes orange at 300°C and also when boiled in a solvent of high boiling point. Sublimes *in vacuo* at 213–215°C. Scarcely soluble in water and in organic solvents, except for dioxane and tetrahydrofuran. Soluble in concentrated H_2SO_4 to give a yellow solution with green fluorescence. Soluble in alkaline water (red-yellow). Ultraviolet absorption spectrum maxima at about 270, 290, 320, 340, and 460 mμ. $C_{23}H_{18}O_6$: C = 70.43%; H = 4.75%; O = 24.25%; four active H = 1.2%; C—CH_3 = 4.4%. Very stable to the action of alkali, acid, and heating. Not a quinone. *Monosodium salt:* Red, water-soluble crystals. *Monostearate:* Yellow rhomboid crystals. M.p. (not sharp) 113°C. Soluble in petroleum ether (1, 3).

Biological activity: Active on *Staph. aureus* and *B. subtilis* at 1: 20,000,000, and on *M. tuberculosis* at 1:500,000 to 1,000,000 (1).

Toxicity: LD_{50} (croceomycin) 20 mg per kg intraperitoneally (4).

References:

1. Brockmann, H. and Schmidt-Kastner, G. Naturwissenschaften **38:** 479–480, 1951.
2. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
3. Brockmann, H. and Schmidt-Kastner, G. Chem. Ber. **87:** 1460–1469, 1954.
4. Shibata, M. *et al.* Ann. Rept. Takeda Research Lab. **16:** 82–87, 1957.

Rhodocidin

Produced by: Streptomyces phoenix.

Method of extraction: Addition of 0.03 per cent H_2O_2 to broth. Filtration of the mycelium. Addition of 15 per cent NaCl; extraction with ethyl acetate. Concentration, addition of petroleum ether, extraction with water. Addition of 2 per cent $NaHSO_4$, washing with ethyl acetate. After addition of 2 per cent acetone, extraction with ethyl acetate. Addition of petroleum ether, extraction with water, concentration, lyophilization.

Chemical and physical properties: Red powder, showing a broad peak of absorption at 500 to 530 mμ. Soluble in water and in many organic solvents. Inactivated rapidly by dilute acids and alkalies. Inactivated by the mycelium of *S. phoenix* in absence of excess oxygen. Organic solvent solutions and dry powders are stable indefinitely at 5°C.

Biological activity: Active against mycobacteria, gram-positive and gram-negative bacteria. Active against *Streptococcus pyogenes* in mice. Much more active by the intraperitoneal route than by the intravenous or intramuscular routes.

Toxicity: LD_{50} (mice) 2.1 mg per kg intraperitoneally, 2 mg per kg intravenously, and 3 mg per kg intramuscularly.

Reference: 1. Charney, J. *et al.* Antibiotics & Chemotherapy **3:** 788–792, 1953.

Rhodomycetin

Produced by: Red mutant of *Streptomyces griseus.*

Remarks: Originally described as "rhodomycin."

Method of extraction: Precipitated from broth with acid. Precipitate extracted successively with methanol. Methanol concentrated *in vacuo* until precipitate forms. Broth also extracted with n-butanol at pH 7.2, then at pH 3.0. Extract washed with water, then extracted with 0.2 *M*

Na_2CO_3 solution acidified to precipitate antibiotic.

Chemical and physical properties: Dark red powder. Gradual darkening but no melting point up to 300°C. Red in acidic solution, blue in alkaline. *Blue form:* Soluble in water, but rapidly destroyed under these conditions. *Red form:* Insoluble in water. Soluble in ethylene glycol, monomethyl ether, ethyl ether, acetone, acetic acid, and various alcohols. Ultraviolet absorption spectrum maxima at 235, 540, and 580 mμ, or at 525 mμ (concentrated H_2SO_4). Reddish violet in concentrated H_2SO_4. Ethanolic solution becomes amber on addition of alcoholic $FeCl_3$. Alcoholic solution stable at 70°C for 10 minutes at pH 4.5 to 9.3. Least stable at alkaline pH. N = >1 per cent (Kjeldahl and Dumas). Reduced by zinc dust with heating in presence of acid or base; cooling restores color, indicator properties, and antibiotic activity. No reaction with zinc dust at room temperature or in absence of acid or base. Decolorized to yellow by sodium hydrosulfite; reversibly oxidized to red by air. Not affected by sodium bisulfite.

Biological activity: Active on gram-positive bacteria. Slightly active on mycobacteria. Not active on gram-negative bacteria or fungi. Not active in mice against *Staph. aureus* infections.

Toxicity: Mice tolerate 25 mg per kg intraperitoneally, and 200 mg per kg subcutaneously.

Reference: 1. Shockman, G. and Waksman, S. A. Antibiotics & Chemotherapy 1: 68–75, 1951.

Rhodomycins

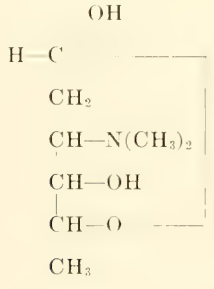
Produced by: Streptomyces purpurascens cultures, which also produce isorhodomycin A (2, 7, 9); *S. cinereoruber* (9).

Remarks: It was found that the biologically active chromophores of the various rhodomycins, as well as other related chromophoric moieties which were biologically inactive, exist in the mycelium and culture-filtrate in greater quantities than the antibiotics themselves (7). It was also found that true *S. purpurascens* strains produce the glycoside of β-rhodomycinon (*i.e.*, rhodomycin A) as the major component, and the glycosides of α-, γ-, δ-, and ε-rhodomycinons in only minor amounts. On the other hand, strains of *S. cinereoruber* produce the glycosides of both β- and γ-rhodomycinons as the major components, with the latter often in greater quantity than the former; α-rhodomycinon is occasionally found in small quantities, but δ- and ε-rhodomycinon are usually absent from these broths (9).

Method of extraction: Rhodomycin complex: I. Concentrated broth-filtrate extracted with ether at pH 3.0. Aqueous phase passed through a Lewatit KSN column (Na^+ form). Eluted with ammonia-methanol. Eluate neutralized, and concentrated *in vacuo*. Residue taken up in water and precipitated as the picrate (7). II. Impurities precipitated from concentrated culture-filtrate by addition of acetone and basic lead acetate. Filtered. Filtrate extracted with butanol at pH 8.6. Re-extracted into 10 per cent acetic acid. Precipitated as the picrate (4). *Rhodomycin A:* Dry, finely pulverized mycelium exhaustively extracted with 0.5 *N* HCl-containing acetone. Extract neutralized, concentrated *in vacuo*, then successively extracted with ether. Aqueous acetone phase adjusted to pH 8.5, treated with a concentrated methanol-lead acetate solution, centrifuged, and the supernatant extracted with chloroform-ethanol (3:1). Extract evaporated *in vacuo*. Residue distributed between butanol and pH 5.8 phosphate buffer to give major Fraction A and minor Fraction B. Fraction A crystallized as red needles from isopropanol-ethanol (1:1) on the addition of a drop of concentrated HCl. Further purification by countercurrent distribution (butanol-pH 6.0 phosphate buffer). Active fraction then separated into rhodomycin A, isorhodomycin A, and a third zone containing rhodomycin B, isorhodomycin B, and rhodomycinons, by ring paper chromatography [(10 per cent acetic acid (stationary phase) and butanol saturated with 10 per cent acetic acid (mobile phase)]. Zones cut apart and eluted with methanol-water. Eluates concentrated *in vacuo*, taken up in chloroform-methanol (4:1) and evaporated *in vacuo*. *Rhodomycin B:* Culture-filtrate adjusted to pH 8.5 and stirred with chloroform-methanol (5:2). Chloroform phase concentrated *in vacuo*. Concentrate extracted with 10 per cent acetic acid. Extract shaken with ether, adjusted to pH 8.5, and treated with lead acetate to precipitate impurities. Filtrate adjusted to pH 8.5 and extracted with chloroform-ethanol (4:1). Purification of concentrated extract by ring paper chromatography (formamide-butanol, 1:1) and enough water to give two phases. The upper phase is mobile; the lower, stationary. Five zones are obtained, corresponding to isorhodomycin A, rhodomycin A, isorhodomycin B, rhodomycin B, and rhodomycinons. Paper strip containing rhodomycin B eluted with chloroform and precipitated as the hydrochloride (3, 6, 7).

Chemical and physical properties: Rhodomycins are glycosides of rhodomycinons α, β, γ, δ, and ε (9). *Complex:* Dark red powder. At pH 8.6 (isoelectric point of the rhodomycin complex), soluble in

lower alcohols, chloroform, ether, and acetone. At other pH values, soluble in water, lower alcohols, and glacial acetic acid. Orange-red at acid pH, purple-red at neutrality, and blue at alkaline pH. Light-absorption maxima at 504, 535, 542, and 575 mμ (benzene) or 567 and 536 mμ (methanol) (1, 4). Irreversibly destroyed in strong base or acid (4). *Picrate:* Yellow-red substance. Soluble in acetone and hot ethanol. Insoluble in water, ether, and benzene. Hydrochloride and phosphate are water-soluble (1, 4). *Perchlorate:* Crystalline substance; m.p. 198°C (decomposition, uncorrected). Soluble in alcohol, acetone, and pyridine. Sparingly soluble in water. In acetic anhydride, gives a yellow-red solution turning bluish violet with red fluorescence upon addition of pyroboric acetate (4). *Rhodomycin A: Hydrochloride:* Red prisms; m.p. 205°C. Soluble in water and lower alcohols. Very slightly soluble in benzene and chloroform. Insoluble in ether and petroleum ether. $[\alpha]^{18}_{506-760} = +178° \pm 10°$ (c = 0.13 per cent in methanol). Ultraviolet light-absorption spectrum maxima at about 230, 260, 295, and 500 mμ (methanol). C = 54.55%; H = 6.82%; O = 26.13%; N = 3.20%; Cl = 9.64%. *Perchlorate:* Fine red needles; m.p. 188°C (6). Mild hydrolysis products of rhodomycin A include "rhodosamine," which is isomeric with "picrocin" from picromycin (5):



Rhodomycin B: Hydrochloride: Red prisms; m.p. 180°C. Soluble in lower alcohols. Moderately soluble in water and acetone. Scarcely soluble in chloroform and benzene. $[\alpha]^{18}_{606-760} = +174° \pm 10°$ (c = 0.05 per cent in methanol). Ultraviolet absorption spectrum maxima at about 230, 260, 295, and 500 mμ. $C_{17-19}H_{23-27}O_{6-7}N \cdot HCl$: C = 54.88%; H = 6.70%; O = 26.02%; N = 3.41%; Cl = 8.94%. *Base:* Amphoteric substance. Soluble in dilute acid; very scarcely soluble in water (6). *Rhodomycinons:* These are the chromophores of the rhodomycins (see "Remarks"). Rf values (benzene-formamide): α, 0.10; β, 0.27; γ, 0.64; δ, 0.77; and ε, 0.85 (9). *β-Rhodomycinon* (chromophore of rhodomycin A): $C_{20}H_{16-18}O_8$ or $C_{23}H_{20-22}O_9$. Mild alkaline hydrolysis yields γ-rhodomycinon, $C_{20}H_{16-18}O_7$ or $C_{23}H_{20-22}O_8$ (8).

Biological activity: Complex: Active on certain gram-positive bacteria, including *Staph. aureus* at $1:2 \times 10^7$, but not on gram-negative bacteria, except *Hemophilus* and *Brucella*. Not active on fungi (1).

References:

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2. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
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Rifomycins

Produced by: Streptomyces mediterranei.

Remarks: This organism produces a mixture of five antibiotics (rifomycins A, B, C, D, and E). Rifomycin B is a stable acidic component; rifomycins A, C, D, and E are neutral or slightly acidic unstable substances.

Method of extraction: Filtration of broth at pH 7.0 to 8.0. Extraction of the broth with ethyl acetate at pH 2.0. Back-extraction in pH 6.5 to 7.5 buffer. The ethyl acetate layer can be concentrated *in vacuo*, and addition of petroleum ether yields crude rifomycin complex containing Fractions A, C, D, and E. The buffer layer is acidified to pH 2.0 and extracted with ethyl acetate. The solvent is concentrated *in vacuo* and rifomycin B crystallizes (1).

Chemical and physical properties: Rifomycin complex: Amorphous brown powder. Soluble in water at pH 9 to 10, giving a dark red solution (maximal solubility, 2 per cent). Soluble in acetone, chloroform, ethyl acetate, methanol, and ethanol. Insoluble in petroleum ether. Solutions of rifomycin complex at pH 6.0 lose 10 per cent of their activity in 24 hours at 5°C, and 75 per cent at 37°C. Very unstable at neutral or alkaline pH. Stability can be improved by addition of ascorbic acid; however, ascorbic acid increases the instability in acidic solutions. The complex can be

separated into four fractions by paper chromatography (3 per cent ammonium chloride-1 per cent ascorbic acid). The complex can also be separated into four components by countercurrent distribution (methanol-0.01 *N* HCl-benzene-petroleum ether, 10:5:15:5) if 100 transfers are used: rifomycin E = tubes 1 to 10; rifomycin D = tubes 40 to 50; rifomycin C = tubes 60 to 70; rifomycin A = tubes 90 to 100. *Rifomycin C and D:* Brown amorphous substances. Soluble in methanol, acetone, chloroform, and ethyl acetate. Slightly soluble in neutral or acidic water. Soluble (2 per cent maximum) in buffer solutions at pH 9 to 10 in presence of ascorbic acid to prevent rapid destruction. Light-absorption maxima at 240, 305, 385, and 461 mμ in methanol or ethyl acetate. The peak at 461 mμ shifts to 422 mμ in the presence of a reducing agent. Positive Tollen, Fehling, and $FeCl_3$ tests. Negative ninhydrin test. Rifomycin C gives a faintly positive ninhydrin test only after strong acid hydrolysis. Rifomycin C: C = 61.52%; H = 6.73%; N = 4.21%. Rifomycin D: C = 62.17%; H = 6.58%; N = 3.53%. *Rifomycin E:* Maximal light absorption at 400 mμ. *Rifomycin B:* Brilliant yellow prismatic needles. If a warm saturated solution is slowly cooled, very long, thin needles are formed. Decomposes at 160–164°C and does not melt until 300°C. Solubility: water, 0.027%; methanol, 2.62%; ethanol, 0.44%; acetone, 0.31%; chloroform, 0.34%; benzene, 0.018%; ethyl acetate, 0.19%; carbon tetrachloride, 0.0011%; ethyl ether, 0.005%; petroleum ether, <0.005%. Dibasic acid. Equivalent weight, 765. C = 61.75%; H = 6.72%; N = 1.88%; O = 29.22%. Proposed empirical formula: $C_{39}H_{51}NO_{14}$. Molecular weight (Rast) 750. $[\alpha]_{589}^{20} = -11°$ (c = 1 per cent in methanol). Light-absorption maxima in phosphate buffer (pH 7.3) at 223, 304, and 425 mμ. Infrared light-absorption spectrum given in reference 1. Crystals are very stable. Increased biological activity of solutions at pH 4.0, resulting from conversion of the antibiotic into a more potent substance. Stability studies carried out by recovering the crystalline antibiotic from solutions showed that at 37°C, after 160 hours at pH 4.0, 12 per cent of the antibiotic is left, and at pH 7.2, 79 per cent of the antibiotic remains undegraded (1).

Biological activity: Rifomycins D and C have more biological activity than rifomycins E and A. *Rifomycin B:* Active *in vitro* against gram-positive bacteria, including mycobacteria; inactive against gram-negative bacteria and fungi. With *Staph. aureus*, no cross-resistance observed between rifomycin B and penicillin, erythromycin, novobiocin, oleandomycin, streptomycin, chloramphenicol, and tetracycline. Serum does not affect the antibiotic activity. Upon standing, solutions show an increase in biological activity, because of the formation of a derivative which is more potent biologically than rifomycin B and which can be separated by paper chromatography. Active in experimental infections (mice) caused by *Streptococcus hemolyticus*, *D. pneumoniae*, and *Staph. aureus* (2). Very active *in vivo* against freshly isolated strains of *Staph. aureus* (4).

Toxicity: Rifomycin B: LD_{50} (mice) 2040 mg per kg intravenously, and >3000 mg per kg intraperitoneally, subcutaneously, and orally. LD_{50} (rats) 1680 mg per kg intravenously, and >3000 mg per kg intraperitoneally, subcutaneously, and orally. LD_{50} (dogs) about 1200 mg per kg intravenously. LD_{50} (guinea pigs) about 3000 mg per kg intraperitoneally. Further pharmacological data in reference 3. Not absorbed orally (4).

Utilization: Rifomycin B: Active intramuscularly against a number of infections in human beings.

References:

1. Sensi, P. *et al.* Antibiotics Ann. 1959–1960, pp. 262–270.
2. Timbal, M. T. Antibiotics Ann. 1959–1960, pp. 271–276.
3. Maffii, G. and Timbal, M. T. Antibiotics Ann. 1959–1960, pp. 277–284.
4. Furesz, S. and Scotti, R. Antibiotics Ann. 1959–1960, pp. 285–292.

Rimocidin

Produced by: Streptomyces rimosus (1, 9). This culture also produces oxytetracycline (9).

Synonym: Antibiotic PA 85 (9).

Method of extraction: I. Broth-filtrate extracted with butanol at pH 9.0. Butanol extracted with dilute mineral acid to remove oxytetracycline. Butanol: (a) treated with active carbon, washed with dilute acids and alkali, then washed with a petroleum fraction (b.p. 30–50°C) to separate out an aqueous layer containing rimocidin; (b) concentrated *in vacuo* at 30–50°C, then treated with petroleum ether. Purified by passage through a column containing an IR-100-IR4B mixture. Crystallized from an alcoholic solution containing triethylamine sulfate upon addition of acetone, then recrystallized from methanol-water (9). II. Adsorbed from broth on activated carbon and eluted with aqueous low molecular weight alcohols or pyridine. Forced into the aqueous layer on addition of hexane or ethyl ether (9).

Chemical and physical properties: Amphoteric

conjugated tetraene. *Sulfate:* Fine hair-like needles or large fragile plates containing 7 per cent water of hydration; m.p. 151°C (decomposition). Ultraviolet absorption spectrum maxima at 279, 291, 304, and 318 mμ (80 per cent ethanol). Infrared spectrum given in reference 9. $[\alpha]_D^{25} = +75.2°$ (c = 1 per cent in methanol. C = 57.65%; H = 7.82%; N = 1.81%; S = 2.03%. Forms salts with metals (1, 9).

Biological activity: Active on filamentous fungi and yeasts, including *Trichophyton* (1). Active on certain protozoa, including *Trypanosoma cruzi* (7) and *Trichomonas vaginalis* (8), and moderately active on *Endamoeba histolytica* and *Leishmania* (4). Not active on bacteria (1). No successful activity *in vivo* has been reported. Active by seed treatment on *Ascochyta pisi* and *Mycosphaerella pinodes* infections of peas, *Colletotrichum lindemuthianum* infection of beans, *Stemphylium* infection of carrots, *Alternaria* infection of radishes (11), and *Pythium debaryanum* and *Rhizoctonia solani* infections of pine and birch (14). Partial control of *Verticillium* infections of potato and tomato, and *Rhizoctonia* infections of lettuce, potato, and tomato (5). Reduction of fungus rots of potato seed pieces (13). Molluscacide (10). Enhances wheat root growth in water solution (3). Increases chick (16) and hog growth rates when added to the diet (2). Slightly active on RC mammary carcinoma in mice (15).

Toxicity: LD_{50} (mice) 30 mg per kg intravenously (1, 4). Toxic to human spermatozoa at 0.0125 μg per ml (6). Nontoxic to carnation cuttings at 120 ppm (12).

References:

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Ristocetins

Produced by: Nocardia lurida (2).

Synonym: Spontin.

Method of extraction: Broth-filtrate treated with carbon. Eluted with 40 per cent acetone (at pH 2.0). Eluates concentrated to 1/10 volume, adjusted to pH 5.0, and chromatographed on carbon. Developed with 16 per cent acetone containing 1 ml per liter of 6 *N* H_2SO_4. Active fractions identified by paper chromatography (80 per cent methanol-20 per cent water containing 1.5 per cent NaCl). In this system, ristocetin A has an Rf of 0.4, and B, 0.15. First fractions contain mainly A; middle fractions A and B; and as the concentration of acetone in the developer increases, the last fractions contain mainly B. Purification by chromatography on alumina after concentration and removal of excess H_2SO_4 with $Ba(OH)_2$. Ristocetins A and B converted to their free bases by passage over mixed Amberlite resins IR-45 (OH^-) and IR-120 (H^+). Crystallized from water and ethanol (1).

Chemical and physical properties: Complex: Amphoteric substances. Soluble in water, dilute acids and alkalies. Insoluble in methanol, ethanol, acetone, ether, dioxane, chloroform, and glacial acetic acid. $\lambda_{max}^{H_2O}$ 280. Absorption increases after inactivation with alkali. Infrared spectrum the same for both A and B (1). Folin-Ciocalteau (for phenols) and anthrone tests positive. Negative biuret, Sakaguchi, and maltol tests. In aqueous solution, pH is 8.0. Isoelectric point is 8.1 (in glycine buffer of ionic strength 0.05). Rf values on paper chromatography given in reference 2. Stable at acid pH; inactivated at pH >7.0. Hydrolysis (acid) products include D-arabinose, glucose, mannose, rhamnose, and a ninhydrin-positive substance (5). *Ristocetin A:* Hexagonal prismatic rods. Crystallographic data given in reference 1. C = 52.5%; H = 5.6%; N = 5.3%; S = 1.0% (sulfate). *Ristocetin B:* No comparable optical crystallographic or roentgen ray data could be obtained on the needle crystals of B. C = 53.2%; H = 5.7%; N = 6.1%; S = 0.4% (sulfate).

Biological activity: Active on gram-positive

bacteria, including mycobacteria, and *Actinomyces bovis*, but not on gram-negative bacteria, yeasts, filamentous fungi, or protozoa. A and B show cross-resistance to each other, but no cross-resistance to erythromycin, penicillin, streptomycin, the tetracyclines, chloramphenicol, or polymyxin B. Development of resistance is slow. Bactericidal. B is about 3 to 4 times more active than A against streptococci *in vitro* and *in vivo*. Active *in vivo* on *Streptococcus pyogenes*, *Staph. aureus*, and *D. pneumoniae*, but not on *Mycobacterium* in mice (1, 4, 5).

Toxicity: LD_{50} (mice) intravenously: ristocetin A sulfate: 1350 to 2000 mg per kg; ristocetin B sulfate: 645 to 760 mg per kg; complex (predominantly A): 888 to 1820 mg per kg. The lower figure in each case represents rapid injection; the higher figure, slow injection. Orally, 5 to 12.5 gm per kg, and subcutaneously, 1 gm per kg of the complex are tolerated by mice (3). Hematologic complications noted during therapy in 8 out of 10 patients tested (4).

Utilization: Enterococcal, staphylococcal, and pneumococcal infections resistant to other antibiotics. Used intravenously only (1, 4, 6, 7).

References:

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2. British Patent 765,886, January 16, 1957.
3. Hwang, K. *et al.* Antibiotics Ann. 1957–1958, pp. 163–179.
4. Gangarosa, E. J. *et al.* New Engl. J. Med. **259:** 156–161, 1958.
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Roseocitrin B

Produced by: Streptomyces roseocitreus (4). This culture also produces roseocitrin A, a substance more closely related to streptothricin (see streptothricins).

Method of extraction: I. Isolation procedure like that of streptomycin (1, 2). II. Broth-filtrate extracted with 1 per cent lauric acid-butanol. Extract re-extracted into water (pH 2.0). Aqueous extract concentrated, shaken with 80 per cent phenol, then with ether. Chromatographed on Al_2O_3 with 50 per cent acetone as solvent and developer. Active fractions dried *in vacuo*, dissolved in methanol, then precipitated with acetone. Precipitated from a methanolic solution with acetone-ether. Purified by conversion to the helianthate, then the hydrochloride (3).

Chemical and physical properties: Basic substance. Soluble in 50 to 60 per cent acetone. Insoluble in ether (2). *Hydrochloride:* Needles. *Helianthate:* d.p. 305–306°C. Positive Molisch, indole, and Elson-Morgan tests. Negative Fehling, maltol, biuret, ninhydrin, xanthoproteic, Adamkiewitz, Liebermann, Neubauer, and Sakaguchi tests (3). Rf values in various systems given in reference 2. Stable to acid (3).

Biological activity: Moderately active (12.5 to 100.0 μg per ml) on gram-positive and gram-negative bacteria and mycobacteria. No cross-resistance with streptothricin or streptomycin. Most active at alkaline pH (3).

Toxicity: Mice tolerate 1 gm per kg intravenously (3).

References:

1. Kato, H. J. Antibiotics (Japan) **3:** 579–581, 1950.
2. Kato, H. J. Antibiotics (Japan) **6B:** 3–8; **6A**:42, 1953.
3. Kato, H. J. Antibiotics (Japan) **6B:** 141–149; **6A**:108, 1953.
4. Kato, H. J. Antibiotics (Japan) **6B:** 205–209, 1953.

Roseomycin

Produced by: Streptomyces roseochromogenes strains (1, 2, 6, 10), and *Streptomyces* sp. (6).

Remarks: Roseomycin is classified as a "streptothricin I"-type antibiotic, according to the terminology of certain Japanese workers. See streptothricin-like antibiotics for an explanation.

Synonym: Antibiotic 36 (4).

Method of extraction: Same as that for streptothricin (1, 2).

Chemical and physical properties: Basic substance. *Reineckate:* Platelets; m.p. 114°C (decomposition) (1, 7). *Helianthate:* Platelets; m.p. 211–216°C (decomposition) (1, 7). *Hydrochloride:* Positive Fehling, Molisch, glucosamine, and Tollen tests (1, 10). Negative biuret, ninhydrin, xanthoproteic, Adamkiewitz, Liebermann, Neubauer, Tollen naphthoresorcinol, indole, maltol, and Sakaguchi tests (7, 10). Boiling at pH 2.0 or 5.0 for 20 minutes causes a 90 per cent loss in activity; at pH 9.0, a 50 per cent loss (10).

Biological activity: See "Remarks" above. More active *in vitro* than streptomycin on *V. cholerae* (2). More active on streptomycin-resistant *E. coli* than on the parent strain (5, 10). Equally active as streptomycin against tularemia and

Eberthella typhosa infections in mice (4, 8). Also active on *Sal. typhi*, *V. cholerae*, and *Pasteurella tularensis* infections in mice (10). Increases growth of chickens when added to the diet (4).

Toxicity: Mice tolerate about 1 gm per kg (HCl salt) intravenously and subcutaneously (1).

References:

1. Kikuchi, M. J. Antibiotics (Japan) **3:** 839–844, 1950.
2. Ishida, N. J. Antibiotics (Japan) **3:** 845–853, 1950.
3. Nagao, I. J. Antibiotics (Japan) 3(Suppl. C): 20–23, 1950.
4. Nagao, I. J. Antibiotics (Japan) **4:** 24–28, 1951.
5. Ishida, N. Tôhoku J. Exptl. Med. **54:** 180, 1951.
6. Kuroya, M. *et al.* Tôhoku J. Exptl. Med. **55:** 209–217, 1952.
7. Kuroya, M. *et al.* Tôhoku J. Exptl. Med. **55:** 289–297, 1952.
8. Miyamori, A. J. Antibiotics (Japan) **5:** 163–165, 1952.
9. Takahashi, T. *et al.* J. Antibiotics (Japan) **6B:** 452–454, 1953.
10. Ishida, N. Tôhoku J. Exptl. Med. **58:** 153–161, 1953.

Roseothricin

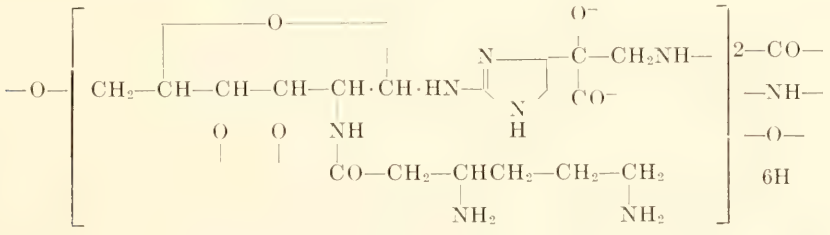
Produced by: Streptomyces roseochromogenes (9).

Remarks: Streptothricin-like substance. Roseothricin A may be identical to streptothricin VI and to part of the mycothricin complex (14) (see streptothricin-like antibiotics).

Synonyms: Antibiotic H 277 (1, 8). Antibiotic H 107 is very closely related to or identical to roseothricin (1).

Method of extraction: Impurities in broth precipitated by acidification. Filtrate adsorbed on charcoal at pH 8.0. Eluted with 10 per cent acidic (H_2SO_4) acetone. Eluate adjusted to pH 4.0. Addition of 0.5 per cent sodium acetate and acetone precipitates the sulfate. Reprecipitated as picrate, which is recrystallized from hot 50 per cent ethanol or methanol. Conversion to hydrochloride. Purification by chromatography on alumina from methanol. Developed with methanol, 80 per cent methanol and water. Concentrated active fractions freeze dried. Converted to helianthate (1). Paper chromatography (butanol-methanol-water containing 3 per cent ammonium reineckate, 4:1:2) indicates a number of components, with Rf values of 0.04, 0.16, 0.33, 0.54, and 0.77 (3, 5). Component A can be separated from B and C by chromatography on Amberlite IRC-50 (Na^+ phase) at pH 7.0 (7) and developed with citric acid-HCl buffer, starting with pH 4.6 and with gradual lowering of the pH by 0.2 unit (9) or with 2 per cent ammonium sulfate solution. Component A can be precipitated as the phosphotungstate (13).

Chemical and physical properties: Basic substance. *Complex:* Helianthate: m.p. 215–218°C. Reineckate: m.p. 185–190°C. Picrolonate: m.p. 225–230°C (decomposition). C = 48.23%; H = 5.63%; N = 20.34%. Positive Elson-Morgan, ninhydrin, biuret, and Fehling tests. Negative Tollen, Pauly, Bial, Nylander, Millon, Trommers, Molisch, Folin, Hopkins-Cole, Sakaguchi, and maltol tests. Inactivated by $KMnO_4$, formalin, and 1.0 *N* methanolic HCl. Partially inactivated by cysteine. Not inactivated by H_2O_2 or hydroxylamine (1, 9). *Component A:* Molecular weight, about 900. pK values of 11.4, 10.6, 9.6, 9.0, 8.3, and 7.2 (10). Diffuses more rapidly than B or C (7). Acid hydrolysis products include β-lysine, "roseonine" (streptolidine), and α-D-glucosamine (8, 9, 11–13). (See Table 43 for Rf values of components.) Partial structure of roseothricin (13):



Biological activity: Complex: Active on gram-negative and gram-positive bacteria. Partial cross-resistance with streptothricin; none with streptomycin (1, 6). Resistance develops slowly (2). Active in mice on *Shigella flexneri*, *Sh. sonnei*, and *Sal. enteritidis* (6). Component A is less active than a B-C mixture (7), but has the same activity qualitatively (6).

Toxicity: Complex: LD_{50} (mice) 14 mg per kg intravenously. *Roseothricin A hydrochloride:* LD_{50}

(mice) 155 mg per kg intravenously. *Roseothricin B and C hydrochloride (mixture):* LD_{50} (mice) 1 to 6 mg per kg intravenously (7, 9).

References:

1. Hosoya, S. *et al.* Japan. J. Exptl. Med. **20:** 121–133, 1949.
2. Hosoya, S. *et al.* Japan. J. Exptl. Med. **20:** 481–488, 1950.
3. Hosoya, S. *et al.* Japan. J. Exptl. Med. **20:** 683–687, 1950.
4. Hosoya, S. *et al.* J. Antibiotics (Japan) **4:** 79–83, 1951.
5. Hosoya, S. *et al.* J. Antibiotics (Japan) **4:** 314–316, 1951.
6. Hosoya, S. J. Antibiotics (Japan) **5:** 248–250, 1952.
7. Saburi, Y. J. Antibiotics (Japan) **6A:** 193, 1953.
8. Nakanishi, N. J. Antibiotics (Japan) **7A:** 174–175, 1954.
9. Nakanishi, N. *et al.* Bull. Chem. Soc. Japan **27:** 539–543, 1954.
10. Hirata, Y. *et al.* Res. 3rd Congr. Intern. Biochem. 87, 1955.
11. Goto, T. *et al.* Bull. Chem. Soc. Japan **30:** 304–305, 1957.
12. Nakanishi, K. and Ohashi, M. Bull. Chem. Soc. Japan **30:** 725–728, 1957.
13. Goto, T. *et al.* Bull. Chem. Soc. Japan **30:** 729–737, 1957.
14. Horowitz, M. I. Thesis, Rutgers University, 1957.

Rotaventin

Produced by: Streptomyces rubrireticuli (3, 6, 7), formerly referred to as *S. reticuli* (3); *S. griseocarneus* (2, 4) (these strains also produce hydroxystreptomycin); *Streptomyces* sp. (5).

Method of extraction: I. Mycelium extracted with methanol or acetone. Extract concentrated *in vacuo*. Concentrate adjusted to pH 2.0 to precipitate antibiotic. Washed with ether, dissolved in methanol, and reprecipitated by addition of water (3). Purified by countercurrent distribution (water-methanol-$CHCl_3$-benzene, 7:23:30:15) (1, 5). Crystallized from water-methanol-benzene (4). Broth extracted with butanol (5).

Chemical and physical properties: White thin leaves or colorless platelets (1, 3). Yellows at 130°C; m.p. 160–175°C (decomposition). Soluble in methanol, butanol, amyl alcohol, and acetone. Insoluble in ether, petroleum ether, chloroform, benzene, ethyl acetate, amyl acetate, trichloroethylene, 5 per cent $NaHCO_3$, and acidic water (pH 2.0). No precipitate in alcoholic solution with phosphotungstic or picric acids, methyl orange, lead acetate, $HgCl_2$, or $CaCl_2$ (1, 3). Gradually yellows in air. Red in concentrated H_2SO_4. $FeCl_3$ test negative (3).

Biological activity: Active on *Sacch. sake*, *A. niger*, and *P. glaucum* at 6 μg per ml. Little or no activity on *C. albicans*, *Mucor*, *Rhizopus*, *Willia*, and *Botrytis*. Not active on bacteria (3). Slightly active on *Trichomonas vaginalis* (6).

Toxicity: LD_0 (mice) about 150 mg per kg intraperitoneally, 770 mg per kg subcutaneously (3).

References:

1. Hosoya, S. *et al.* Japan. J. Exptl. Med. **21:** 279–283, 1951.
2. Benedict, R. G. *et al.* J. Bacteriol. **62:** 487–497, 1951.
3. Hosoya, S. *et al.* J. Antibiotics (Japan) **5:** 451–453, 1952.
4. Hosoya, S. *et al.* Japan. J. Exptl. Med. **22:** 23–24, 1952.
5. Taguchi, H. J. Fermentation Technol. **32:** 195–199, 1954.
6. Hosoya, S. *et al.* J. Antibiotics (Japan) **6A:** 92–97, 1953.
7. Hosoya, S. Quoted in Benedict, R. G. Botan. Rev. **19:** 229–320, 1953.

Rubidin

Produced by: Streptomyces sp.

Method of extraction: Acidification of broth-filtrate and refiltration. Clear red filtrate shaken with diethyl ether or n-butanol until the aqueous layer shows removal of red pigment. Extract evaporated *in vacuo*. Purified by countercurrent distribution (diethyl ether-aqueous HCl, pH 4.0).

Chemical and physical properties: Acid-base indicator (blue at alkaline pH, red at acid pH). Dark red amorphous powder. Insoluble in petroleum ether; slightly soluble in $NaHCO_3$ solution; highly soluble in most organic solvents. Stable at acid pH; unstable at pH 8 or above. Contains no N. C = 51.9%; H = 5.56%; O = 42.54%. Ultraviolet absorption spectrum maxima at 500 and 530 mμ (0.1 *N* HCl); 400 and 520 mμ (0.1 *N* NaOH); and 320, 415, and 490 mμ (n-butanol). Infrared spectrum data given in reference 1. Positive alcoholic $FeCl_3$ test. Reversibly reduced, changing to colorless compound, with sodium bisulfite or with zinc dust at alkaline or acid pH. Rf = 0.80 by one-dimensional paper chromatography (dioxane-water, 1:1). Distinguished by paper chromatography from anthocyanins and rhodomycins.

Biological activity: Active mainly on gram-positive bacteria. Moderately active on *Pr. vulgaris*

(20 μg per ml); slightly active (100 μg per ml) on *Ps. aeruginosa*. Not active on *A. niger*.

Reference: 1. Banerjee, A. K. *et al.* Antibiotics Ann. 1955–1956, pp. 640–647.

Rubromycin

Produced by: Streptomyces collinus. This organism also produces collinomycin (1).

Method of extraction: Finely ground dried mycelium washed with petroleum ether, ether, or carbon disulfide. Exhaustively extracted with 80 per cent acetone-2 *N* H_2SO_4 (100:12), or acetone, chloroform, methylene chloride, ethyl acetate, tetrahydrofuran, or dioxane. Acetone extract adjusted to pH 6.0, concentrated *in vacuo*, and residue shaken with water and ether. Most of the antibiotic precipitates at the interface. Precipitate centrifuged, washed with water, and dried. Taken up in methylene chloride, and shaken with saturated $NaHCO_3$ solution to precipitate colored impurities at the interface. Methylene chloride layer neutralized, washed with water, and concentrated to dryness. Residue purified by chromatography on activated calcium sulfate from chloroform. Crystallized from chloroform, methanol, or ethyl acetate.

Chemical and physical properties: Red, thin, long, rectangular crystals, m.p. 125°C (decomposition) (from methanol or ethyl acetate). Crystals appear pale violet microscopically. Crystallizes from chloroform-methanol as orange prisms; m.p. 280–282°C (uncorrected). Moderately soluble in acetone and chloroform. Slightly soluble in ether and lower alcohols; almost insoluble in water, petroleum ether, and $NaHCO_3$. In 2 *N* NaOH and in concentrated H_2SO_4, gives a reddish violet coloration, with ultraviolet absorption spectrum maxima at 546 and 584 mμ, and 518 to 520 mμ, respectively. Red-violet Na salt. Decolorized by sodium hyposulfite, but not by sodium bisulfite. Color returns on exposure to air. A pyridine-methanol solution treated with titanium trichloride gives a blue-green solution changing to red-violet. No N. C = 60.30%; H = 4.26%; O = 33.91%. *Acetate:* Yellow needles; m.p. 193–195°C (uncorrected). Heating in methylene chloride yields a biologically active decomposition product having red crystals, m.p. 265–268°C (uncorrected), similar to rubromycin, and having ultraviolet absorption maxima (ether) at 532 and 547 mμ (2, 3).

Biological activity: Most active on gram-positive bacteria; less active on gram-negative bacteria (2).

References:

1. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
2. Brockmann, H. and Renneberg, K. H. Naturwissenschaften **40:** 59–60, 1953.
3. Brockmann, H. and Renneberg, K. H. German Patent 918,162, September 20, 1954.

Ruticin

Produced by: Streptomyces sp. closely related to *S. rutgersensis*.

Method of extraction: Addition of 0.03 per cent H_2O_2 to culture, filtration, and extraction of culture-filtrate with ethyl acetate at pH 4.0. Back-extraction with water at pH 8.0. Extraction and counter-extraction repeated with increasingly smaller volumes of ethyl acetate and water. From the final aqueous extract, the antibiotic precipitated out by adjusting the pH to 4.0. Crystalline material obtained by repeated crystallization of crude ruticin from methanol.

Chemical and physical properties: The purest sample of ruticin was composed of orange needle-like crystals, which could be transformed into an inactive product crystallizing in rosettes. Acidic substance. Soluble in methanol, ethanol, and ethyl acetate. The sodium salt is soluble in water, methanol, and ethanol; insoluble in ethyl acetate. Ultraviolet absorption maxima at 262 and 364 mμ. The free acid is unstable. In presence of living cells, ruticin is destroyed in absence of an excess of oxygen.

Biological activity: Active against certain gram-positive and gram-negative bacteria.

Toxicity: LD_{50} (mice) 20 mg per kg intravenously.

Reference: 1. Fisher, W. P. *et al.* Antibiotics Ann. 1953–1954, pp. 174–176.

Sarcidin

Produced by: Streptomyces achromogenes.

Method of extraction: Adsorption on charcoal at pH 7.0. Elution with acidic alcohol, concentration *in vacuo*, crystallization at 0–5°C, recrystallization from 80 per cent aqueous ethanol.

Chemical and physical properties: m.p. 274–275°C (decomposition). C = 41.9%; H = 5.0%; N = 21.8%. Halogen and S may also be present. Maximal stability at pH 2.0.

Biological activity: Active against *Sarcina lutea* at 1 to 1.5 μg per ml. No activity against a large number of bacteria and fungi tested.

Toxicity: LD_0 (mice) >660 mg per kg intraperitoneally.

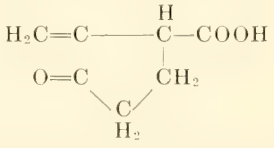
Reference: 1. Takeuchi, T. *et al.* J. Antibiotics (Japan) **6A:** 31–32, 1953.

Sarkomycin

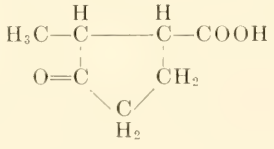
Produced by: Streptomyces erythrochromogenes (3).

Method of extraction: Extracted from broth-filtrate at pH 2.0 with butanol or ethyl acetate. Back-extraction into water at pH 7.0. Adsorption on charcoal at pH 2.0, and elution with 80 per cent ethanol at pH 7.0 (2), or extraction from water into methyl isobutyl ketone at pH 2.5 to 3.0. Purification by any of the following: treatment with magnesol to remove pigment and impurities; partition chromatography on silica gel (chloroform-pH 6.0 phosphate buffer); adsorption from neutral aqueous solution (I) on Dowex 18X (Cl^-) resin and elution by gradient technique with 0.1 to 1 *M* NaCl; adsorption from I on Amberlite IR-411 (OH^-) and IR-120 (H^+) mixture and elution with 1.5 per cent NaCl; or countercurrent distribution (ethyl acetate-pH 4.8 phosphate buffer). In countercurrent distribution, 50 transfers give a single major peak with a distribution coefficient (d.c.) = +2.4 and a minor peak (d.c. = 11), a decomposition product. Freed from β-sarkomycin by extraction of I with benzene at low pH. Extract chromatographed on Florisil and eluted with dioxane. β-Sarkomycin was not purified (9).

Chemical and physical properties: Free acid: Oil. Soluble in water, methanol, ethanol, butanol, and ethyl acetate. Sparingly soluble in petroleum ether. Almost insoluble in acetone, butyl acetate, and ether (2, 8). Red or reddish pink color with anthrone (9, 10). Negative biuret, Sakaguchi, xanthoproteic, Molisch, Fehling, Benedict, Tollen,



Scheme 1. Sarkomycin: 2-methylene-3-oxocyclopentanecarboxylic acid.

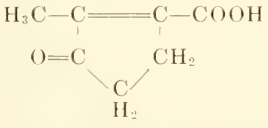


Scheme 2. Dihydrosarkomycin: 2-methyl-3-oxocyclopentanecarboxylic acid.

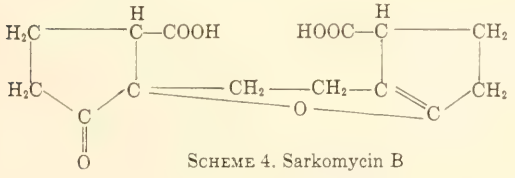
Seliwanoff, Foulgen, and picric acid tests. Positive nitroprusside and iodine azide tests (for SH groups) reported for the crude antibiotic (2) were shown to be caused by ethyl acetate-soluble impurities (4). Noncrystalline inactive precipitate with 2,4-dinitrophenylhydrazine. Pure samples have an ultraviolet absorption spectrum maximum at 230 mμ; crude samples have only end-absorption or a shoulder at 250 to 270 mμ (9). Infrared spectrum given in reference 9. Paper chromatography (10 per cent Na citrate (pH 5.5)-water-saturated CCl_4-isoamyl alcohol, 1:2) indicates two components: Rf = 0.6 (major component, sarkomycin) and Rf = 0.85 (β-sarkomycin) (9). Occasionally, three components (23) have been observed (pH 6.5 buffer, water-saturated butanol). Hydrogenation destroys antibacterial activity and the anthrone reaction, but not antitumor activity, yielding a white crystalline acid, dihydrosarkomycin: $C_5H_{10}O_3$ (2-methyl-3-oxocyclopentanecarboxylic acid); m.p. 99–99.5°C. $[\alpha]_D^{25}$ = +66.7° (c = 1 per cent in water) (Scheme 2). Permanganate oxidation product is succinic acid. Strong heating *in vacuo* gives a crystalline rearrangement product, $C_7H_8O_3$ (2-methyl-3-oxo-1-cyclopentanecarboxylic acid), m.p. 175–176°C, optically inactive and devoid of biological activity (Scheme 3). Sarkomycin is 2-methylene-3-oxocyclopentanecarboxylic acid (Scheme 1) (9). Both d- and l-forms of sarkomycin have been synthesized (27, 30). Polymerizes in presence of peroxide to form high molecular weight polymers (m.p. 280°C) which are biologically inactive (28). Stored at 37°C, Na sarkomycin decomposes to form sarkomycin B (Scheme 4). Sarkomycin and sarkomycin B decompose (in presence of formic acid) to form sarkomycin E (sarkomycin E "Z-crystal") (35) (Scheme 5) (24, 33). Lyophilization of sarkomycin or sarkomycin E gives rise to an inactive product, "M-crystal" (Scheme 6) (33). Sarkomycin reacts with H_2S to give sarkomycins S_1 and S_2 (Schemes 7 and 8) (21, 22, 25) and the less well described S_3 factor (21, 36).

Biological activity: Sarkomycin: Very slightly active on certain gram-positive bacteria, such as *Staph. aureus*, *Streptococcus hemolyticus*, *Micrococcus flavus*, and *B. anthracis;* not active on *B. subtilis*, gram-negative bacteria, *Nocardia*, or *C. albicans* (2, 8, 10, 13). Antistaphylococcal activity parallels antitumor activity (2). Has antitrichomonal activity (21). Bacteriostatic effect lowered by cysteine, glutathione, or serum, but not by methionine. Cysteine and serum have no effect on the antitumor activity of the Na salt, only the Ca salt. Ca salt has less antibacterial

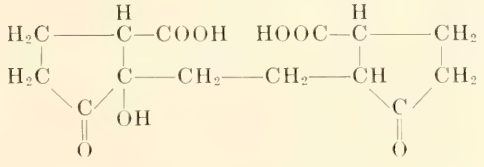
activity than the Na salt (4, 8). *In vivo:* Active on Yoshida sarcoma (*in ovo* and rats), Ehrlich carcinoma (ascitic and solid forms) in mice, increasing survival time and lowering transplantability of tumors (2, 13). Active on sarcoma 180 (ascitic form, mice), Usubuchi sarcoma (rats) (13), Takeda sarcoma (rats), ascites hepatoma 7974 (rats) (16), carcinoma 1025, Krebs 2 ascites carcinoma, Harding-Passey melanoma (moderate activity) (26), RC mammary adenocarcinoma (mice) (37) and Crabb hamster sarcoma (34). Histologically, incidence of mitosis is decreased (11). Destroys tumor cells at both resting and dividing stages (15). Causes desquamation and loss of transferability in HeLa cells, an effect not reversed by cysteine (13). Synthetic d- and l-forms of sarkomycin have the same antitumor activity as the dl-form against Ehrlich ascites carcinoma (mice), but lower antibacterial activity (27, 30). *Sarkomycin B:* Weak or no antibacterial activity; some antitumor activity (33). *Sarkomycin E:* active on cancer cells, *Staph. aureus*, and *Micrococcus flavus* (35). "*M crystal*": Biologically inactive. *Sarkomycin* S_1 *:* Has 10 per cent of the activity of sarkomycin on *Staph. aureus*, equal activity on *Micrococcus flavus*, greater activity on *C. albicans*, no antitrichomonal activity, 25 per cent of the anti-HeLa cell activity, and 50 per cent of the antitumor activity (21, 25). *Sarkomycin* S_2 *:* Has 60 per cent of the anti-*Micrococcus* activity of sarkomycin, and slight antitumor activity (21).



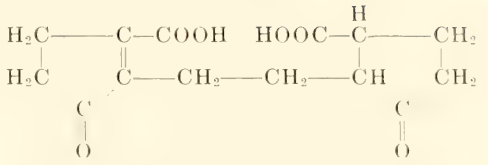
Scheme 3. Sarkomycin rearrangement product: 2-methyl-3-oxo-1-cyclopentanecarboxylic acid.



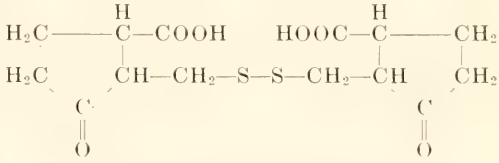
Scheme 4. Sarkomycin B



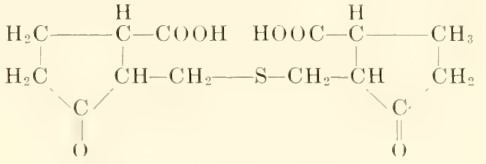
Scheme 5. Sarkomycin E



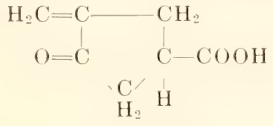
Scheme 6. M-crystal



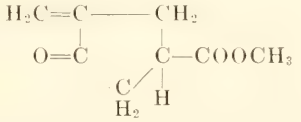
Scheme 7. Sarkomycin S_1



Scheme 8. Sarkomycin S_2



Scheme 9. Analogue a: 5-methylenecyclopentanone-3-carboxylic acid.



Scheme 10. Analogue b: methyl-5-methylenecyclopentanone-3-carboxylate.

Sarkomycin S_3 *:* Has 220 per cent of the antimicrococcal activity of sarkomycin, increased anti-*Candida* activity, moderate antitumor activity, but no antitrichomonal activity (21, 22, 36). *Dihydrosarkomycin:* No antibacterial or anti-HeLa cell activity. Active on Ehrlich ascites carcinoma (25).

Toxicity: Sarkomycin: LD_{50} (mice) 800 to 1600 mg per kg intravenously, 400 to 800 mg per kg subcutaneously, and 1 to 1.8 gm per kg intraperitoneally (4, 11). Mice tolerate 4.8 gm per kg orally (8). Addition of glutathione, cysteine, or serum reduces toxicity (4). Na salt causes necrosis at site of subcutaneous administration; Ca salt

less (6). *Sarkomycin E:* LD_{50} (mice) >600 mg per kg (no route given) (33).

Utilization: Certain Japanese reports indicate successful use in a variety of neoplastic conditions (5, 7, 12, 14). Others failed to find any objective effect on neoplasms and reported severe side effects, including venous irritation, emesis, and nausea (17, 32).

Sarkomycin Analogues and Derivatives

Produced by: Synthesis.

Chemical and physical properties: Analogues: (a) 5-Methylenecyclopentanone-3-carboxylic acid: Colorless needles; m.p. 67–74°C. Ultraviolet absorption spectrum maximum at 231 mμ (ethanol) (31). Infrared spectrum given in reference 31 (Scheme 9). *(b) Methyl-5-methylenecyclopentanone-3-carboxylate:* Colorless crystals; m.p. (HCl salt) 143–143.5°C (decomposition) (31) (Scheme 10).

Derivatives: (a) Isoniazid-sarkomycin: Red substance. More stable than sarkomycin (28). *(b) Nicotinic acid hydrazide:* Less stable than sarkomycin (28).

Biological activity: Analogues a and *b* are active on *Staph. aureus.* Analogue *a* has antitumor activity on HeLa cells, and prolongs survival time of mice with Ehrlich ascites carcinoma (31). *Derivative a* has antibacterial and antitumor activity (28). *Derivative b* is reputed to be "biologically active" (28).

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Seligocidin

Produced by: Streptomyces sp. resembling *S. roseochromogenes.*

Method of extraction: Mycelium extracted with acetone; evaporated *in vacuo*. Residual aqueous solution extracted with butanol; evaporated to dryness *in vacuo*. Extraction with methanol; slow evaporation *in vacuo*.

Chemical and physical properties: Almost colorless crystalline powder. Sakaguchi and ninhydrin tests positive; biuret test negative. Soluble in methanol; sparingly soluble in ethanol and water. Ultraviolet maximum at 304 mμ in ethanol.

Biological activity: Active against yeasts and filamentous fungi, *S. griseus*, and *Nocardia asteroides*. At higher concentrations, active on *Staph. aureus*, *Sarcina lutea*, and several *Bacillus* species.

Toxicity: LD_{50} (mice) 200 mg per kg intraperitoneally.

Reference: 1. Nakamura, S. *et al.* J. Antibiotics (Japan) **7A:** 57–59, 1954.

Sistomycosin

Produced by: Streptomyces viridosporus (1).

Method of extraction: Broth-filtrate extracted with n-butanol. Extracts concentrated almost to dryness in the dark *in vacuo* at <45°C. Residue washed with chloroform, then extracted with water. Water-extract freeze dried to give sistomycosin. Water-insoluble residue extracted with chloroform containing 10 per cent methanol. Extract chromatographed on Florisil or Decalso and developed with chloroform containing increasing amounts of methanol. Eluted with methanol. Methanol evaporated to dryness *in vacuo*. Residue extracted with water. Freeze dried.

Chemical and physical properties: Neutral tetraene. Microcrystalline, buff to light yellow substance. Browns at 130°C but does not melt below about 230°C. Very soluble in water and methanol. Moderately soluble in "moist" acetone containing not more than 5 per cent water, and aqueous mixtures of aliphatic alcohols containing more than one carbon atom. Practically insoluble in nonpolar solvents. Positive $KMnO_4$, Benedict (boiling), and Molisch tests. Negative Beilstein (for halogen) and $FeCl_3$ tests. Gives a deep cherry-red to chocolate color in H_2SO_4. No color in warm concentrated HNO_3 or HCl saturated with thymol. Ultraviolet absorption spectrum maxima (water) at 218, 292.5, 306, and 320 mμ. Infrared spectrum given in reference 1. Photo-labile. Most stable at pH 7 to 9. Moderately stable to heat. Contains C, H, O, and N.

Biological activity: Active on yeasts and filamentous fungi. Not active on *Cryptococcus neoformans*, bacteria, or actinomycetes (1).

Toxicity: LD_{50} (mice) 90 mg per kg intravenously (1).

Reference: 1. British Patent 712,547, July 28, 1954.

Speciomycin

Produced by: Streptomyces sp. (1).

Synonym: Antibiotic 190/1.

Method of extraction: Adsorbed from broth on activated carbon. Eluted with acidic methanol. Extract concentrated and acetone added to give a precipitate. Taken up in methanol, and solvent removed *in vacuo* (2).

Chemical and physical properties: Light brown, odorless, amorphous powder. Soluble in water and lower alcohols. Insoluble in higher alcohols, acetone, and chloroform. Ultraviolet absorption spectrum maximum at 273 mμ (c = 1 per cent in water) or 270 mμ (c = 1 per cent in 0.1 *N* HCl). No characteristic peak in NaOH. Most stable at acid or neutral pH; unstable at acid pH (2).

Biological activity: Active on gram-positive bacteria; very slightly active on gram-negative bacteria. Inactive on mycobacteria and fungi (1).

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Spiramycins

Produced by: Streptomyces ambofaciens (1, 12) (also produces netropsin (congocidin)); *Streptomyces* sp. resembling *S. ambofaciens* (13).

Synonyms: Foromacidins (13, 16), sequamycin (12), antibiotic R. P. 5337, rovamycin, provamycin, selectomycin.

Remarks: A fourth component, D, not present in the spiramycin broths, was reported in the broths of the foromacidin-producer. This is described below.

Method of extraction: I. Broth-filtrate at pH 9.0 extracted with methyl ethyl ketone, or methyl isobutyl ketone, chloroform, butanol, benzene, or ethyl acetate. Back-extracted into water at pH 2 to 3. Aqueous extract concentrated at pH 6.5 and extracted with chloroform or benzene at pH 9.0. Extract concentrated *in vacuo*, n-butanol added, and pH adjusted to 5. Concentrated again and ether added to precipitate crude spiramycin (4, 12). II. Broth-filtrate extracted with ethyl acetate at pH 8.5. Extract concentrated, then back-extracted into 0.5 *N* aqueous acetic acid. Re-extracted into ethyl acetate at alkaline pH, and extract evaporated to dryness *in vacuo*. Purified by countercurrent distribution (chloroform- 0.2 *N*

citrate buffer, pH 5.0). Active fractions extracted with chloroform at alkaline pH, concentrated, and resubjected to countercurrent distribution. Active fractions crystallized from ethyl ether-petroleum ether to give the complex (27). Components separated by (a) countercurrent distribution between an aromatic hydrocarbon (*e.g.*, benzene) or a chlorinated solvent (dichloroethane) and a phosphate buffer (pH 6 to 7). The aqueous phase of the active fractions adjusted to pH 9 to 10, and the benzene layer separated and distilled off *in vacuo*. Spiramycins II and III are crystallized from benzene; (b) chromatography on alumina. To obtain spiramycin I, column washed with benzene to elute II and III, then I eluted with benzene-acetone, benzene-ethanol, ethanol, or acetone. Spiramycins II and III are separated by elution of III from the alumina column with cyclohexane-benzene or cyclohexane-ether. II is then eluted with ether or ether-acetone; (c) fractional crystallization from benzene (20).

Chemical and physical properties: Macrolide antibiotics (16). Basic substances (19). *Complex:* Cream-colored amorphous substance with a bitter taste. Soluble in chloroform, chlorinated solvents, alcohols, hexane, benzene, ketones, ethyl and amyl acetate. Negative Sakaguchi, Molisch, ninhydrin (before and after hydrolysis), Fehling, biuret, and maltol (before and after hydrolysis) tests. Violet-pink in sulfuric or hydrochloric acids. $[\alpha]_D^{20} = -72°$ (c = 2 per cent in ethanol). Ultraviolet absorption spectrum maximum at 231 mμ ($E_{1\,cm}^{1\%}$ 340). Contains three or four components (depending on producer). Spiramycins II and III differ only by the esterification of the hydroxyl group of I by acetic and propionic acid, respectively (4, 12, 16). *Spiramycin I:* m.p. 133–137°C. Solubility same as that of complex. Ultraviolet absorption spectrum maximum at 232 mμ ($E_{1\,cm}^{1\%}$ 322). Infrared absorption spectrum given in references 12 and 19. $[\alpha]_D^{20} = -96°$ (c = 1 per cent in methanol); −57° (c = 1 per cent in $CHCl_3$). Rf = 0.04 on paper chromatography (paper impregnated with Na_2HPO_4 buffer at pH 9.0; with the light phase of cyclohexane-methyl isobutyl ketone-water, 85:15:25, as developer). $pK_b = 7.7$. C = 60.8%; H = 8.7%; O = 28.5%; N = 3.1%. $C_{45}H_{78}O_{15}N_2$ (12, 16, 19, 20). *Spiramycin II:* m.p. 130–133°C. Same solubility as I. Ultraviolet absorption spectrum maximum at 232 mμ ($E_{1\,cm}^{1\%}$ 307). Infrared spectrum given in reference 12. $[\alpha]_D^{20} = -80°$ (c = 1 per cent in methanol). $pK_b = 7.6$. C = 60.7%; H = 9.0%; O = 26.8%; N = 3.1%. Rf = 0.15 (same system as for I). $C_{47}H_{80}O_{16}N_2$ (16, 19, 20). *Spiramycin III:* m.p. 128–131°C. Same solubility as I. Ultraviolet absorption spectrum maximum at 232 mμ ($E_{1\,cm}^{1\%}$ 327). Infrared spectrum given in reference 12. $[\alpha]_D^{20} = -85°$ (c = 1 per cent in methanol). $pK_b = 7.6$. Rf = 0.22 (see system under I). C = 61.0%; H = 8.5%; O = 26.7%; N = 3.0%. $C_{48}H_{82}O_{16}N_2$ (16, 19). *Foromacidin D:* Colorless powder; m.p. 135–140°C. Ultraviolet absorption spectrum maximum at 231 mμ ($\log \epsilon = 4.44$). Infrared absorption spectrum given in reference 13. $[\alpha]_D = -75°$ (c = 0.81 per cent in ethanol). C = 59.85%; H = 8.48%; N = 3.35% (13). Spiramycins lose biological activity on acid hydrolysis. Products include α- and β-mycarose (dimethyl-4,6 trihydroxy-2,4,5 tetrahydropyran), also isolated from carbomycin, and neospiramycins I, II, and III. *Neospiramycin I:* m.p. 119–120°C. $[\alpha]_D^{20} = -57°$ (c = 1 per cent in methanol). $C_{38}H_{66}O_{12}N_2$. *Neospiramycin II:* m.p. 116–119°C. $[\alpha]_D^{20} = -56°$ (c = 1 per cent in methanol). $C_{40}H_{68}O_{13}N_2$. *Neospiromycin III:* m.p. 117–118°C. $[\alpha]_D^{20} = -52°$ (c = 1 per cent in methanol). $C_{41}H_{70}O_{13}N_2$ (16). The neospiramycins, on further acid hydrolysis, give rise to the "forocidins" (13, 17, 18). *Forocidin A:* $C_{30}H_{51}O_{11}N$. *Forocidin B:* m.p. 236–238°C. $C_{32}H_{53}O_{12}N$. *Forocidin C:* m.p. 218–220°C. $C_{33}H_{55}O_{12}N$. Neospiramycin hydrolysis also yields dimethylamino-5-methyl-6-hydroxy-2-tetrahydropyran (17, 18), the formula of which is given in Chapter 6. Both the neospiramycins and forocidins have an ultraviolet absorption spectrum maximum at 232 mμ (16, 17). Like the spiramycins and the neospiramycins, the forocidins differ in the esterification of the hydroxyl group of A with acetic acid (B) and propionic acid (C) (18). On further hydrolysis, the forocidins give rise to mycaminose (dimethylamino-4-methyl-6-trihydroxy-2,3,5 tetrahydropyran), which has also been isolated from carbomycin (18).

Biological activity: Complex: Active on gram-positive bacteria. Slight to no activity on mycobacteria, gram-negative bacteria, and fungi (4, 6, 12, 13). Most active at alkaline pH. Less active *in vitro* than carbomycin or erythromycin, but equally active *in vivo* (8). Partial cross-resistance with picromycin, carbomycin, and erythromycin (4, 13), but this is not always found clinically (15). *In vivo:* Active on infections (mice) caused by *Diplococcus*, *Streptococcus*, *Staphylococcus*, *D. pneumoniae*, *Pasteurella*, and *Borrelia* (4, 6, 12, 13). Active on psittacosis, *Rickettsia orientalis* (11, 22), and other rickettsiae (rabbits) (2). Antitoxoplasmic activity in rabbits (3, 5). Active on *Plasmodium berghei* (9) and *Endamoeba histolytica* in mice (13). Inactive (mice) on trypanosomes (9). Spiramycins I, II, and III do not differ markedly in their activity (15). Spiramycin activity *in vivo* is

greater than that expected from *in vitro* tests, and may be accounted for by the very high concentrations attained in the tissues (25).

Toxicity: Complex: LD_{50} (mice) 1.25 to 2.5 gm per kg subcutaneously (6, 9, 12, 19), 168 mg per kg intravenously, and 5 gm per kg orally (6, 9). LD_{50} (rats) 9.4 ± 0.8 gm per kg orally. LD_{50} (dogs) 5.2 ± 1.6 gm per kg orally (21). Spiramycins I, II, and III do not differ in toxicity (6, 9). Gastrointestinal disturbances occur in some patients (21).

Utilization: Staphylococcal infections resistant to other drugs. Pneumococcal infections, whooping cough, urethritis (15, 23), ocular and other infections (23, 24). Low serum concentrations reported following administration (7). Active on coccidiosis (*Eimeria tenella*) in chickens. Malarial infections (14). Possible use in amoebic dysentery (10).

Spiramycin Derivatives

Produced by: Hydrogenation of spiramycins (19). Derivatives of spiramycins.

Method of production of neospiramycins: Spiramycins are kept at 20–50°C in water, ethanol, or an ethanol-water mixture at pH 3 to 5 until the biological activity against *B. subtilis* becomes constant. Neospiramycins I, II, and III are thus formed from spiramycins I, II, and III, respectively. Separated by extracting with a mixture of isopropyl ether-isopropanol-buffer (pH 8) or benzene-buffer (pH 5 to 8) mixture, or by chromatography on alumina (26).

Chemical and physical properties: Hydrogenation products: Dihydrospiramycin I: m.p. 128–132°C. $[\alpha]_D^{27} = -83°$ (c = 1 per cent in methanol). *Tetrahydrospiramycin I:* m.p. 132–135°C. $[\alpha]_D^{27} = -79°$ (c = 1 per cent in methanol). *Tetrahydrospiramycin II:* m.p. 125–128°C. $[\alpha]_D^{27} = -63°$ (c = 1 per cent in methanol). *Tetrahydrospiramycin III:* m.p. 135–140°C. $[\alpha]_D^{27} = -71°$ (c = 1 per cent in methanol). All the tetrahydro derivatives have a light-absorption spectrum maximum at 820 mμ. More highly hydrogenated products are inactive biologically (19).

Biological activity: Tetrahydro derivatives have less activity than the parent compounds on *Staph. aureus, K. pneumoniae, D. pneumoniae,* and *Corynebacterium pseudodiphtheriticum,* but more on *B. cereus, Micrococcus citreus, Sarcina lutea,* and *Streptococcus viridans.* Equally active as the parent compounds on *Streptococcus* and *D. pneumoniae* infections in mice (19). *Neospiramycins:* More active than spiramycins on certain organisms (26).

Toxicity: Hydrogenation derivatives: LD_{50} (mice) 1.35 gm per kg subcutaneously (19). *Neospiramycins:* Less toxic than spiramycins (26).

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Staphylomycin

Produced by: Streptomyces virginiae (3).

Synonyms: Antibiotic 899. Factor M_I of staphyl-

omycin believed to be the same as mikamycin (4). Related to streptogramin and antibiotics PA 114A and B (2). M_I is identical with PA 114A and E 129A. Component S differs from E 129B and PA 114B (6).

Method of extraction: Broth-filtrate extracted with chloroform. Extract concentrated. Inactive precipitate formed on addition of petroleum ether; precipitate discarded. Petroleum ether concentrated and residue dissolved in aqueous methanol. On acidification to pH 3.0 with HCl, the antibiotic precipitates (1). Separation into components M_I and M_{II}, and S by chromatographing a chloroform-benzene (1:1) solution of the crude antibiotic on silica gel. Development with chloroform gives five zones: (1) narrow brown, (2) broad yellow, (3) narrow brown, (4) broad tan, and (5) dark brown. Fractions containing zones 1, 2, and 3 are dissolved in hot methanol to give crystals of component S. Fractions containing the first half of zone 4 are dissolved in hot acetone. Component M_I precipitates on addition of petroleum ether; recrystallized from acetone or ethanol-water. Component M_{II} is contained in fractions from zone 5 and the last half of zone 4, along with M_I and pigmented impurities; M_{II} was not purified.

Chemical and physical properties: Crude staphylomycin: Light tan powder; m.p. 138–140°C (decomposition); sinters at 130°C. $[\alpha]_D = -124°$ (c = 0.5 per cent in ethanol). N = 7.98%. Most stable to boiling at neutrality (2). Distinguished from streptogramin by paper chromatography (stationary phase: propylene glycol; mobile phase: benzene) (1). *Factor S:* Weak acid. White needles; m.p. 240–242°C; sinters at 165–167°C. If kept for a few minutes in a bath at 170–175°C, a biologically active substance showing only the 240–242°C melting point is obtained. N = 7.98%. $[\alpha]_D = -28.0°$ (c = 1.0 per cent in ethanol). Ultraviolet absorption maxima at 207 ($E^{1\%}_{1\,cm}$ 590) and 304 mμ ($E^{1\%}_{1\,cm}$ 86) in methanol. Infrared spectrum given in reference 2. Very soluble in chloroform and dimethyl formamide. Soluble in ether to 0.1 per cent; methanol, 0.5 per cent; ethanol, 1.5 per cent; benzene, 2.5 per cent; acetone and ethyl acetate, 3 per cent; and dioxane, 4 per cent. Insoluble in water and petroleum ether. More stable than M_I. At 37°C for 24 hours an inactive yellow precipitate is formed. Addition of 0.1 per cent H_2O_2 prevents formation of the precipitate, but not loss of activity. $pK_a = 9.0$ in ethanol, or 7.7 in dimethylformamide-water (1:2). $C_{38\text{-}39}H_{47\text{-}48}N_6O_9$: C = 62.80%; H = 6.57%; N = 11.47%; O = 19.43%; C—CH_3 = 4.36%; N—CH_3 = 2.37%. No alkoxyl groups. Brown-red color in $FeCl_3$ test. Positive ninhydrin test. Negative 2,4-dinitrophenylhydrazine, Tollen, Fehling, $KMnO_4$, Br in CCl_4, biuret, Molisch, and Ehrlich tests. Hydrolysis products include threonine, α-aminobutyric acid, and proline, and possibly norvaline and phenylalanine. The chemical structure of factor S has recently been elucidated (7). *Factor M_I :* Neutral substance. Light tan powder; m.p. 165–167°C (decomposition). $[\alpha]_D = -190° \pm 2°$ (c = 0.5 per cent in ethanol); $-174° \pm 2°$ (c = 0.5 per cent in methanol). Ultraviolet absorption spectrum maximum at 216 mμ ($E^{1\%}_{1\,cm}$ 582) and an inflection at 270 mμ ($E^{1\%}_{1\,cm}$ 200) (methanol). Very soluble in chloroform and dimethylformamide. Approximate solubilities (per cent): ether, 0.1; benzene, 0.3; ethyl acetate, 0.5; acetone, 2; methanol and ethanol, 4; dioxane, 5. Insoluble in water and petroleum ether. $C_{28}H_{36}N_3O_8$: C = 61.99%; H = 6.75%; N = 7.59%; O = 23.52%; C—CH_3 = 7.09%; N—CH_3 = 2.73%. No alkoxyl groups; two carbonyl groups. Molecular weight, 555 to 590. No acetylable hydroxyl groups. Green color with $FeCl_3$ test. Positive 2,4-dinitrophenylhydrazine, Tollen, Fehling, Schiff (weak), $KMnO_4$, Br in CCl_4, and Ehrlich tests. Negative Angeli-Rimini, $Fe(OH)_2$, biuret, ninhydrin, and Molisch tests. Hydrogenation products include at least two having no biological activity and no Ehrlich reaction. Hydrolysis products include glycine and proline. Aromatic ring is present (2). *Factor M_{II} :* Related to M_I. Positive Ehrlich reaction (2).

Biological activity: Active on gram-positive bacteria and mycobacteria. Not active on gram-negative bacteria or *C. albicans* (1). Factor S has a synergistic action with M_I and M_{II}. Factors M_I and M_{II} are more active on *Staph. aureus* and *Sarcina lutea* than is S. S is 3 times more active on *B. subtilis* than M_I. M_I is active *in vitro* at 1.2 μg per ml on *M. tuberculosis* H37Rv. No activity on *M. tuberculosis* H37Rv infections in guinea pigs (3). Active in mice on *Streptococcus pyogenes* (1). Factor S is not synergistic with penicillin, erythromycin, streptomycin, or chlortetracycline. M_{II} reduces the activity of M_I on staphylococci. Antibiotic PA 114A activity is enhanced by S and slightly decreased by M_I. PA 114B enhances the activity of M_I and S. The activity of M_I + S is greater than that of PA 114A + PA 114B. Cross-resistance exists between staphylomycin, PA 114A and B, and streptogramin.

Toxicity: Crude staphylomycin: 50 mg per kg tolerated by mice (1). *Purified staphylomycin:* Nontoxic applied topically to human beings. Toxicity reputedly low in experimental animals (5).

Utilization: Staphylococcal infections resistant to other antibiotics (5).

References:

1. DeSomer, P. and Van Dijck, P. Antibiotics & Chemotherapy **5:** 632–639, 1955.
2. Vanderhaeghe, H. *et al.* Antibiotics & Chemotherapy **7:** 606–614, 1957.
3. Van Dijck, P. *et al.* Antibiotics & Chemotherapy **7:** 625–629, 1957.
4. Arai, M. *et al.* J. Antibiotics (Japan) **11A:** 21–25, 1958.
5. DeSomer, P. and Van der Voorde, H. Antibiotic Med. & Clin. Therapy **4:** 786–797, 1957.
6. Ball, D. *et al.* Biochem. J. **68:** 24, 1958.
7. Vanderhaeghe, H. and Parmentier, G. J. Am. Chem. Soc. **82:** 4414–4422, 1960.

Streptimidone

Produced by: Streptomyces rimosus f. *paromomycinus,* which also produces paromomycin.

Method of extraction: Extraction of the culture-filtrate at pH 5.0 with ethyl acetate. Washing of the concentrated extract with dilute aqueous sodium carbonate, dilute acid, and water. Precipitation of the crude streptimidone from ethyl acetate with petroleum ether. Chromatography of the precipitated crude antibiotic on activated carbon from acetone solution. Crystallization from an acetone-isopropanol-ether solution.

Chemical and physical properties: Fine colorless needles; m.p. 72–73°C. Soluble in methanol, ethanol, acetone, ethyl acetate, and chloroform. Slightly soluble in water and ethyl ether. Insoluble in petroleum ether. Most stable at pH 4.5. Destroyed by exposure to sunlight. $C_{16}H_{23}NO_4$. $[\alpha]_D^{28} = +238°$ (c = 0.5 per cent in water). $[\alpha]_D^{27} = +245°$ (c = 0.5 per cent in chloroform). Light-absorption maxima at 232 and 291 mμ (methanol); 231 and 289 mμ (water). Infrared absorption spectrum given in reference 1. Bromine and aqueous permanganate solutions decolorized. Positive m-phenylenediamine test for α,β-unsaturated aldehydes and ketones. Positive sodium nitroprusside test for methyl ketones. Negative ninhydrin, periodate, Tollen, and titanium chloride tests. Structure determined (see Chapter 6). Streptimidone is 3(2-hydroxy-7 methyl-5-methylene-4-oxo-6 nonenyl) glutarimide (1). A somewhat different formula has recently been proposed (3).

Biological activity: Active against yeasts, filamentous fungi, and protozoa. Slight activity against certain bacteria. Has herbicidal activity. Effective against amebic dysentery in dogs.

Toxicity: LD_{50} (mice) 192 mg per kg intravenously. Chronic toxicity in animals is high.

References:

1. Frohardt, R. P. *et al.* J. Am. Chem. Soc. **81:** 5500–5506, 1959.
2. Kohberger, D. L. *et al.* Antibiotics & Chemotherapy **10:** 9–16, 1960.
3. van Tamelen, E. E. *et al.* J. Am. Chem. Soc. **82:** 2974, 1960.

Streptin

Produced by: Streptomyces sp. resembling *S. reticulus-ruber* (2).

Synonym: Similar to streptothricin (1, 2).

Method of extraction: Like that for streptothricin (1, 2).

Chemical and physical properties: Basic substance. Soluble in water, acid, alcohol, and dilute acids. Insoluble in ether, chloroform, and acetone. Thermostable (2).

Biological activity: Qualitatively the same as streptothricin, but more active on a weight basis (2).

References:

1. Woodruff, H. B. and Foster, J. W. J. Bacteriol. **52:** 502, 1946.
2. British Patent 644,582, October 11, 1950.

Streptocardin

Produced by: Streptomyces sp. and *Nocardia* sp.

Method of extraction: Extraction of the culture-filtrate with ether at pH 4.0. Back-extraction in pH 7.0 phosphate buffer. Extraction in ether at pH 4.0. Concentration of ether *in vacuo*; residue dissolved in acetone; concentration to dryness. Crystallization from ether solutions.

Chemical and physical properties: Acidic substance, soluble in many organic solvents. Brown needles. Alkali salts are soluble in water. Ultraviolet light-absorption maxima at 365 mμ ($E_{1\,cm}^{1\%}$ 187) with a shoulder at 242 to 252 mμ.

Biological activity: Active against gram-positive bacteria, including mycobacteria, and gram-negative bacteria. No activity against the fungi tested.

Toxicity: LD_{50} (mice) 12.5 mg per kg intravenously.

Reference: 1. Fisher, W. P. *et al.* Antibiotics Ann. 1953–1954, pp. 177–178.

Streptocin

Produced by: Streptomyces griseus strains. The original streptocin-producer formed other antibiotics, including what was believed to be cycloheximide, as well as an antibiotic which was highly active on *Sarcina lutea.* Streptocin is also formed by strains of *S. griseus* that produce grisein and streptomycin (1, 2).

Method of extraction: I. Mycelium extracted with water-saturated diethyl ether, methanol, ethanol, acetone, or water. Solvent evaporated to dryness. Residue taken up in ethanol or ethyl acetate, then concentrated to dryness. Residue taken up in petroleum ether and chromatographed on alumina. Developed with solvents of increasing polarity, finally eluted with ethanol-methanol (1:1), followed by 1 per cent glacial acetic acid in methanol. Streptocin crystallizes on standing or on concentration of solvent. Can also be extracted from broth-filtrate (1). II. Ether-extract of mycelium sublimed at 100°C at 1×10^{-5} mm. Recrystallized from 1 per cent glacial acetic acid in methanol or acetone (3).

Chemical and physical properties: Acidic substance. Colorless needles in rosettes. Loses crystalline structure on standing. Can be sublimed. Soluble in polar solvents such as water, methanol, and ethanol; insoluble in nonpolar solvents such as chloroform. Infrared spectrum given in reference 3. Stable to changes in pH and heat. Contains C, H, and O (1, 3).

Biological activity: Slightly active on gram-positive bacteria, including mycobacteria. Limited antifungal activity. Active on *Trichomonas* (1, 3).

Toxicity: Reputed to have extremely low toxicity intravenously (mice).

References:

1. Waksman, S. A. *et al.* Proc. Soc. Exptl. Biol. Med. **70:** 308–312, 1949.
2. Kupferberg, A. B. *et al.* J. Bacteriol. **59:** 523–526, 1950.
3. British Patent 677,743, August 20, 1952.

Streptogramin

Produced by: Streptomyces graminofaciens (4).

Synonyms: Said to contain certain components which act synergistically, like those of the antibiotics PA 114, E 129, staphylomycin, and mikamycin complexes (6).

Method of extraction: Broth-filtrate extracted with ethyl acetate. Ligroin added to extract to give active precipitate. Dilute solution of precipitate in methanol treated with excess 1 per cent phosphate buffer (pH 7.0) at 5°C to precipitate impurities. Concentration of supernatant *in vacuo* gives streptogramin.

Chemical and physical properties: Complex: Substantially neutral, noncrystalline substance; m.p. about 155°C. Soluble in methanol, ethanol, acetone, and ethyl acetate; sparingly soluble in water and dilute acid; insoluble in ligroin. Ultraviolet absorption spectrum maximum at 225 mμ, with inflections at 270 to 290 mμ and 350 mμ (solvent not given). Infrared spectrum data given in reference 1. $[\alpha]_D^{25} = -134°$ (concentration and solvent not given). Positive test with dimethylaminobenzaldehyde in H_3PO_4 (for ring nitrogen) and $FeCl_3$ test. Negative ninhydrin test. Inactivated by alkali above pH 9.5. C = 62.25%; H = 6.62%; N = 8.42%. $C_{26}H_{33}O_7N_3$ (1).

Biological activity: Active mainly on gram-positive bacteria, including mycobacteria. Active on *Hemophilis.* Little or no activity on most gram-negative bacteria, fungi and *Trichomonas vaginalis.* Development of resistance of *Staph. aureus* is slow. Cross-resistance with erythromycin, carbomycin, oleandomycin, and spiramycin. Active in mice on *Diplococcus, Pasteurella,* β-hemolytic *Streptococcus,* and *Staphylococcus* infections (1–3, 5).

Toxicity: LD_{50} (mice) 450 mg per kg intraperitoneally (1).

References:

1. Charney, J. *et al.* Antibiotics & Chemotherapy **3:** 1283–1286, 1953.
2. Verwey, W. F. *et al.* Bacteriol. Proc. 79, 1954.
3. Jones, W. F. *et al.* Proc. Soc. Exptl. Biol. Med. **93:** 388–393, 1956.
4. British Patent 776,035, 1957.
5. Verwey, W. F. *et al.* Antibiotics & Chemotherapy **8:** 500–505, 1958.
6. Woodruff, H. B. Quoted in Watanabe, K. *et al.* J. Antibiotics (Japan) **12A:** 112–113, 1959.

Streptolins

Produced by: Streptomyces sp. (1), similar but not identical to the strain of *S. lavendulae* that produces antibiotic 136 (5); *S. griseus* f. *farinosus* (11).

Remarks: Broth contains streptothricin and at least two other antibiotics in addition to streptolins A and B; the same five components are found in broths containing antibiotic 136 (5). Other workers have reported five components in the streptolin complex (9, 10). Rf values of the components in streptolin broths are given in Table 43 of "Streptothricin-like Antibiotics."

Method of extraction: I. Broth-filtrate treated with Darco G-60 at acid pH, filtered, then adsorbed on a Darco G-60-Hyflo Super-Cel mixture. Eluted with acidic acetone. Addition of acetone to active fractions produces a gum, which is taken up in water, adjusted to pH 2.0, and precipitated with methanol. Recrystallized as reineckate. Purification by conversion to other salts (5). Repeated

recrystallization of the helianthate of the complex gives streptolin A (5).

Chemical and physical properties: Streptolin complex: Basic substance. *Sulfate:* White amorphous powder. *Helianthate:* Crystalline; m.p. 203°C (decomposition) (5). *Hydrochloride:* $[\alpha]_D^{28} = -22°$ (c = 1.8 per cent in water) (1). Positive biuret, ninhydrin, Shaffer-Somogyi (copper reagent), and Park-Johnson ferroferricyanide tests (4). Negative Sakaguchi, maltol, phenylhydrazine, and 2,4-dinitrophenylhydrazine tests (1, 4). Maximal stability at pH 3 to 3.5 when heated to 120°C for 1 hour. Less stable in more alkaline or acidic solutions. Diffuses poorly in agar (1). $C_{17}H_{31-33}N_5O_8(HCl)_2$ or $C_{24}H_{45-47}N_7O_{11}$: C = 39.95%; H = 6.66%; N = 13.65% (4). Contains no N-alkyl functions (3). *Streptolin A:* Isomeric with streptolin B. *Helianthate:* Crystalline; m.p. 206°C (decomposition). *Sulfate:* Amorphous. $[\alpha]_D^{25} = -20°$ (c = 1 per cent). Infrared spectrum given in reference 5. *Streptolin B:* Acid hydrolysis products include L-β-lysine, "streptolidine," and 2-amino-2-desoxy-α,D-gulose (α-D-gulosamine) (6, 8). (See schemes with streptothricins.)

Biological activity: Complex: Active on gram-positive and gram-negative bacteria (1). Streptolin B is more active than A (5). Partial cross-resistance with streptothricin and, in one case, streptomycin (2). Active on the nematode *Rhabditis briggsae* (7).

Toxicity: Complex and streptolin A: 6 mg per kg injected intravenously is lethal to mice (5).

References:

1. Rivett, R. W. and Peterson, W. H. J. Am. Chem. Soc. **69:** 3006–3009, 1947.
2. Pagano, J. F. *et al.* Proc. Soc. Exptl. Biol. Med. **79:** 359–363, 1952.
3. van Tamelen, E. E. and Smissman, E. E. J. Am. Chem. Soc. **74:** 3713–3714, 1952.
4. Smissman, E. E. *et al.* J. Am. Chem. Soc. **75:** 2029–2031, 1953.
5. Larson, L. M. *et al.* J. Am. Chem. Soc. **75:** 2036–2039, 1953.
6. van Jamelen (*sic*), E. E. Quoted in Nakanishi, K. *et al.* Bull. Chem. Soc. Japan **27:** 539–543, 1954.
7. Gochnauer, M. B. and McCoy, E. J. Exptl. Zool. **125:** 377–406, 1954.
8. van Tamelen, E. E. *et al.* J. Exptl. Zool. **78:** 4817–4818, 1956.
9. Dyer, J. R. Quoted in reference 8.
10. Horowitz, M. I. Thesis, Rutgers University, 1957.
11. Hall, H. H. and Benedict, R. G. U. S. Patent 2,846,310, August 5, 1958.

Streptolydigin

Produced by: Streptomyces lydicus (2). This culture also produces actithiazic acid.

Synonym: Portamycin.

Method of extraction: I. Broth adjusted to pH 7.5 to 8.0, heated to 60°C for 10 minutes, cooled to 27.4°C, filtered. A. Filtrate extracted at pH 7.5 to 8.0 with methylene chloride. Extract concentrated until it contains 150 mg of solids per ml, then stirred with pH 4.0 citrate buffer. Addition of Skellysolve B to the methylene chloride causes the free acid form of streptolydigin to precipitate. Precipitate leached with Skellysolve B and acetone (3:1), and resulting solution concentrated to an oil. Oil dissolved in acetone. Addition of water precipitates the antibiotic. Countercurrent distribution indicates a biologically inactive product (1, 2). B. Broth-filtrate extracted with methylene dichloride at pH 3.0. Extract stirred with water at pH 7.0; solvent removed by vacuum distillation. Aqueous residue freeze dried. Purified by extraction with ethylene dichloride at pH 2.5 and concentrated *in vacuo*. Oily residue taken up in ethyl ether and precipitated with Skellysolve B (2). II. Can also be isolated by adsorption on IRC-50 (Na^+ form), Dowex 1, or Permutit DR, and elution with aqueous neutral or acidic alcohols (2).

Chemical and physical properties: Strong enol acid. Orthorhombic needles. Loses birefringence at 110°C and melts at 144–150°C. Soluble in common polar organic solvents. Insoluble in water and dilute mineral acids (<1 mg per ml). Ultraviolet absorption spectrum maxima at 234 ($E^{1\%}_{1\,cm}$ 139.1), 357.5 ($E^{1\%}_{1\,cm}$ 590.5), and 370 mμ ($E^{1\%}_{1\,cm}$ 560.3), with inflections at 295, 309.5, and 342 mμ (0.01 *N* ethanolic sulfuric acid); or at 261 ($E^{1\%}_{1\,cm}$ 223.6), 291 ($E^{1\%}_{1\,cm}$ 270.7), and 336 mμ ($E^{1\%}_{1\,cm}$ 331), with an inflection at 302 mμ (0.01 *N* ethanolic KOH). Infrared spectrum given in references 1 and 2. $[\alpha]_D^{25} = -64°$ to $-67°$ (c = 2 per cent in 0.005 *N* KOH in 95 per cent ethanol); or $-93°$ (c = 1.6 per cent in chloroform). Positive $FeCl_3$, Br_2 in CCl_4, iodoform, and titanium trichloride (for enediols and enols of 1,3-diketones) tests. Negative bromine water, Fehling (Benedict modification), Molisch, 2,6-Dichlorophenol-indophenol reduction, biuret, and ninhydrin tests. No free amino groups. Crystallographic data given in references 1 and 2. Most stable at alkaline pH; less stable at pH 5.0; unstable at more acidic pH. Loses 50 per cent of activity in a 50 per cent ethanolic solution at 25°C. Stable for 4 months at 4°C. $pK_a = 5.3$ (in 65 per cent ethanol), or 5.7 (in 67 per cent dimethylformamide). $C_{33}H_{47}O_9N_2$ (2) or $C_{32-35}H_{46-50}N_2O_{9-10}$ (1): C = 64.29%; H = 7.68%; N = 4.70%; C—

CH_3 = 9.5% (2). Forms a biologically inactive, yellow addition product with bromine in CCl_4 : $C_{33}H_{47}O_9N_2Br_3$ (2) or $C_{32}H_{45}O_9N_2Br_3$ (1). Addition product has no definite melting point, chars at >100°C, gives positive $FeCl_3$ test, and has two weak maxima in ultraviolet light at 255 and 290 to 295 mμ (1, 2). *Calcium salt:* Soluble in alcohol and methylene chloride. Insoluble in water and hydrocarbons. *Na salt:* Buff-colored powder. Soluble in water, ethanol, and chloroform, but not in hydrocarbons. *K salt:* Same solubility as Na salt (1, 2). Infrared spectra of these salts given in reference 2.

Biological activity: Active on gram-positive bacteria, including *B. cereus*, *Corynebacterium diphtheroides*, *D. pneumoniae*, *Erysipelothrix rhusiophathiae*, *Listerella monocytogenes*, clostridia, mycobacteria, streptococci, and *Nocardia asteroides*. Less active on micrococci. Not active on gram-negative bacteria, except *Pasteurella multocida*. Not active on fungi or viruses. Optimally active at pH 7.0. Partially bound by serum; not affected by cysteine, methionine, or penicillinase. Effective against *Str. hemolyticus*, *D. pneumoniae*, and *P. multocida* infections in mice. Not effective in *K. pneumoniae*, *Sal. typhi*, or *Sal. paratyphi* B infections (1).

Toxicity: LD_{50} (mice) 533 mg per kg intraperitoneally (in 1 per cent sodium carboxymethylcellulose) (1).

References:

1. DeBoer, C. *et al.* Antibiotics Ann. 1955–1956, pp. 886–902.
2. British Patent 779, 570, July 24, 1957.

Streptomycin

Produced by: Streptomyces griseus (135), *S. bikiniensis* (13), *S. olivaceus* (36), *S. mashuensis* (82), *Streptomyces* sp. resembling *S. roseochromogenes* (65), and *Streptomyces* sp. resembling *S. olivaceus* (52).

Synonym: Streptomycin A.

Method of extraction: I. Adsorbed from broth on IRC-50 or other weak carboxylic acid cation exchange resin at pH 7.0. Resin washed with water, then 1 per cent citric acid, and adjusted to pH 6.0 with triethylamine, monoethanolamine, or similar amines, and eluted with hydrochloric or sulfuric acid. The amine displaces contaminating metal ions and is eluted as the sulfate with streptomycin, but does not precipitate from the eluate with streptomycin on addition of methanol (118). Metallic contaminants can also be removed from the eluate without the use of amines by a sequestering agent such as ethylenediaminetetraacetic acid. The antibiotic is re-adsorbed on IRC-50 at pH 6.8 and eluted with sulfuric acid at pH 2.0. Eluate neutralized with IR4B resin, deionized on a "monobed" of IR-120-IR4B, and freeze dried (113). Another process uses resins IRC-50, XE 89, and Dowex 50X-16 in succession; the first two to absorb the antibiotic, the last to remove impurities (120). II. Whole broth acidified with HCl or H_2SO_4 to pH 2 to 4, stirred with charcoal, and filtered. Filtrate neutralized (pH 6 to 8) and adsorbed on charcoal. Charcoal washed with water, aqueous methanol or ethanol, and eluted with 0.8 *N* formic acid in methanol-water (1:1), 0.1 *N* methanolic HCl, dilute acids, 60 per cent aqueous acetone containing HCl, or 5 to 10 per cent acetone-water containing H_2SO_4 (pH 2.5). Eluates give a precipitate of streptomycin on addition of acetone to 75 per cent, or can be neutralized, concentrated under reduced pressure, and taken up in methanol, followed by acetone precipitation. Purified by chromatography on alumina (sulfuric acid-washed) at pH 5 to 6, or on Darco G-60. Methanol or aqueous methanol is used as solvent and developer. Eluates concentrated to remove the methanol, then lyophilized. Further purification by salt conversion (picrate → phosphotungstate → sulfate/helianthate → trihydrochloride; or trihydrochloride → $CaCl_2$ complex) (4, 7, 9, 15, 25). IIIA. Streptomycin is quantitatively precipitated from broths with orange II (sodium *p*-(2-hydroxy-1-naphthylazo)benzene sulfonate) at pH 5.5, or with Naphthol blue-black (disodium salt of 8-amino-7-*p*-nitrophenylazo-2-phenylazo-1-naphthol-3,6-disulfonic acid). Such precipitates can be converted (40, 41) to useful salts, such as the sulfate, by suspending them in alcohols or Cellosolves, or aqueous mixtures of these, treating the suspension with an amine salt (*e.g.*, triethylamine sulfate) to give a precipitate of streptomycin sulfate from the anhydrous solvents. (Acetone must be added to aqueous mixtures to give a precipitate.) IIIB. Wetting agents, such as the "Tergitols" (sodium salts of synthetic higher aliphatic alcohols), "Duponols" (partial sulfuric acid esters of higher aliphatic alcohols), aromatic sulfonic acids, sulfonated oils, and "Ultrawet," precipitate streptomycin from broths or partially purified solutions. For example: Tergitol 7 (sodium sulfate derivative of 3,9-diethyltridecanol-6) in amyl or butyl alcohols is added to streptomycin-containing solutions at pH 3.3 to 7.5 to give precipitated streptomycin "tergitate." Crystallized from methanol-water. Aging for >8 days increases the yield of streptomycin inorganic salts because of tautomerism (see "Chemical and Physical

Properties"). At this point the organic solution of the streptomycin-wetting agent salt may be: (a) chromatographed on acid-washed alumina at pH 4.0 and eluted with 0.1 *N* HCl in anhydrous methanol; (b) transformed to the *trihydrochloride* or *trinitrate* by adding the concentrated acid of the salt desired, or a methanolic solution thereof, along with the calcium salt of the acid. Trihydrochloride is recrystallized from aqueous solution on addition of methanol or methanolic $CaCl_2$; or (c) transformed to the *sulfate* or *phosphate* formed by adding the appropriate aqueous acid or amine salt thereof. The sulfate crystallizes out of the aqueous phase. The phosphate precipitates from the organic phase on addition of methanol (39, 59, 60). IIIC. Precipitated as the pentachlorophenate, taken up in methanol, and filtered. Fractional precipitation as the sulfate. Fractionally precipitated from an aqueous solution of the pentachlorophenate with aqueous sodium pentachlorophenate or as the double pentachlorophenate-sulfate salt (74). IV. Broth-filtrates or aqueous solutions containing streptomycin adjusted to pH 12, solid NaCl added, and the whole extracted with 2-aminoheptane or other liquid primary alkyl or aralkyl amines. Extract concentrated *in vacuo*, filtered, and water and chloroform or amyl acetate added, the water to extract the streptomycin, the chloroform or amyl acetate to extract the amine. Aqueous extract washed with $CHCl_3$ and acetone, acidified, and dried (37). V. Separated from mannosidostreptomycin (occurring with streptomycin in broths) by (a) the formation of the streptomycin-$CaCl_2$ complex (mannosidostreptomycin does not form this complex); (b) countercurrent distribution using an aqueous phase containing 0.5 per cent $NaHCO_3$ and 1.0 per cent NaCl, and a solvent phase containing Pentasol (mixed synthetic amyl alcohols) and 5 per cent stearic acid. Active fractions agitated with 7 *N* H_2SO_4 and benzene to transfer streptomycin to the aqueous phase (23). Other systems include: aqueous 0.125 *M* phosphate-0.375 *M* borate buffer (pH 7.15) *versus* 15 per cent lauric acid in Pentasol (24). VI. Purified by stirring an aqueous solution with phenol-CCl_4 (7:3) at pH 9.5. Back-extracted from solvent layer into dilute H_2SO_4 at pH 6.5 (74).

Chemical and physical properties: Strongly basic substance (25). *Base:* Amorphous, light yellow powder (9). Positive Sakaguchi, Fehling (boiling), hydroxyl group, and ammoniacal $AgNO_3$ tests. Negative nitrous acid, ninhydrin, Hopkins-Cole, Millon, xanthoproteic, biuret, and Pauly diazo tests (4, 18). Gives a garnet-red color with cold alkaline aqueous sodium nitroprusside, which becomes slowly pink, then yellow, on standing or heating. Color is yellow in acetic acid (18). Stable to moderately acidic and alkaline solutions. Maximal stability at pH 3 to 7 at <25°C. Solutions of the sulfate show a 50 per cent loss of activity at 95°C for 4½ hours (25). Aqueous acid hydrolysis yields *streptidine:* optically inactive needles, $C_8H_{18}N_6O_4$. Streptidine sulfate: prisms; dipicrate: needles or stout prisms. Both decompose with charring at >250°C. Streptomycin reacts with bromine water to give biologically inactive *streptomycinic acid*, a light pink-colored amorphous product (containing 5 per cent moisture). Sinters at 226°C; m.p. 231°C (decomposition). $[\alpha]_D^{25} = -92°$ (c = 1.52 per cent in water) (5, 11). Treatment of streptomycin with methanolic HCl yields a derivative (I) of *streptobiosamine*, methyl streptobiosaminide dimethylacetal, as well as *streptidine*. Strong acid hydrolysis of I decomposes the *streptose* moiety to yield a pentaacetyl derivative of N-methyl-L-glucosamine. Mild alkaline hydrolysis products of streptomycin include the γ-pyrone maltol formed from the *streptose* moiety. Catalytic hydrogenation yields *dihydrostreptomycin* (*q.v.*). A resumé of the key steps in the determination of the structure of streptomycin is given in reference 25, as well as in the references concerning degradative studies. $C_{21}H_{39}N_7O_{12}$. Structural formula given in Chapter 6. The difficulty experienced in obtaining crystalline salts of streptomycin is attributed to the fact that it exists in two tautomeric forms. It is believed that only the form containing a free aldehyde group may be crystallized as an inorganic acid salt. The amorphous and crystalline forms of the various salts of pure streptomycin differ very little in physical properties (59). *Trihydrochloride* (dihydrate): Monoclinic prisms. Decomposes gradually without melting (17). Soluble in water and methanol. Slightly soluble in ethanol (15). Insoluble in ether, chloroform, butanol, pyridine, and acetic acid (4, 15). Only end-absorption in ultraviolet light (below 230 mμ) (2, 4). $[\alpha]_D^{26.6} = -86.1°$ (c = 1 per cent in water) (17). Crystallographic data given in reference 55. *Trinitrate:* Regular tetrahedrons. Decomposes gradually without melting (59). *Sesquisulfate:* Rectangular prisms. Soluble in water. Insoluble in butanol-saturated water and methanol (59, 83). *Calcium chloride complex* (streptomycin trihydrochloride-calcium chloride double salt): Elongated needles; m.p. 200–230°C (decomposition). $[\alpha]_D^{25} = -76°$ (c = 1 per cent in water) (3, 25). *Helianthate:* Hydrate: Dark red crystals (9). Anhydrous: Amorphous coppery powder. Darkens at 205°C and melts at 220–226°C (decomposition) (2). C = 50.34%; H =

5.71%; N = 14.56%; S = 5.76% (9). *Reineckate:* Small needles (4). *Sesquiphosphate:* Hexagonal prisms (59). *Thiosemicarbazone 3HCl:* Colorless crystals; m.p. 205°C (54). *Reineckate-sulfate double salt:* Long, thin plates; m.p. 162–164°C (corrected) (1, 10), 160–163°C (corrected) (7), or 164–165°C (corrected) (4). *Sulfate-sulfite double salt:* Fiber-like colorless crystals. Decomposition begins at about 138°C, resulting in loss of the H_2SO_4 with final decomposition at 170°C without melting (72). Streptomycin also forms a number of double salts with *pentachlorophenate* (73), but attempts to form other double salts with a number of other anions failed (58). "*Tergitate*" (see IIIB under "Methods of Extraction"): Crystals; m.p. 141–142°C (decomposition). Soluble in alcohols. Insoluble or soluble with difficulty in other organic solvents. Addition of 5 to 10 per cent water increases solubility in such solvents (59). Streptomycin forms an oxime (25) and a salt with orange II (2, 40).

Streptomycin Chelates

Streptomycin forms chelates with metals. These are soluble in acetic acid-water; slightly soluble in water, ethanol, diethyl ether, and glacial acetic acid; and insoluble in propylene glycol. They are stable to boiling. All are less biologically active than streptomycin. *Streptomycin-copper:* Pale blue substance. Decomposes at 165–170°C. *Streptomycin-cobalt:* Blue-green substance. Decomposes at 145–150°C (93).

Streptomycin Derivatives

Biologically active, but more toxic derivatives of streptomycin (certain N-alkyl streptomycylamines) have been reported (31).

Biological activity: Active on gram-negative and gram-positive bacteria, including mycobacteria, *A. bovis*, *Nocardia*, and certain spirochetes. Not active on fungi (25). Has antialgal activity (63). Interferes with the killer action of *Paramecium aurelia* (var. 4, stock 51 killer, mating type VII) (44). Increases the rate of division and length of *Tetrahymena pyriformis*. The effect on rate of division is also produced by streptobiosamine, streptamine, streptidine, and guanidine (76). Inactivates phages of *E. coli* and *Staph. aureus*. Most active at a weakly alkaline pH (25). Resistance to streptomycin develops rapidly *in vitro* and *in vivo*. In pathogens, resistance is not accompanied by decreased virulence. Cross-resistance exists with dihydrostreptomycin, mannosidostreptomycin, and others of this group, as well as with streptothricin, neomycin, streptolin, and others (53). Microorganisms dependent on streptomycin have been reported (25). Streptomycin derivatives have been reported that can replace streptomycin for a dependent strain of *Staph. aureus*. Certain of these same derivatives do not support the growth of a dependent strain of *E. coli* but actually inhibit it, and some inhibit a streptomycin-resistant strain of *Staph. aureus* (22). An extract of the cells of a streptomycin-sensitive *E. coli* can replace streptomycin for a dependent strain of *E. coli*. This extract also acts to promote the early growth of the sensitive strain from which it is obtained (126).

Effects on Morphology

Certain gram-negative bacteria, such as *Shigella* and *Salmonella*, became elongated, swollen, and more refractory to staining when exposed to slightly bacteriostatic concentrations. *Sal. typhimurium* assumes a coccoid form. Gram-positive bacteria show no change, although mycobacteria were reported to show increased granulation, decreased length, and loss of acid-fastness (25).

Reversal of Activity

Activity on *Aerobacter aerogenes* can be reversed by several purine and pyrimidine derivatives. A variety of salts reduce the activity of streptomycin *in vitro*, as do glucose, cysteine, and other substances (25, 57).

Activity in Vivo

Active on *M. tuberculosis* (mice, guinea pigs, chick embryos), *Pasteurella pestis* (mice and guinea pigs), *P. tularensis* (mice), *P. multocida* (turkeys), *H. influenzae* (mice), *H. ducreyi* (rabbits), *K. pneumoniae* (mice), *Sal. schottmuelleri* (mice), *Sal. enteritidis* (mice), *Sal. pullorum* (chicks), *Sal. typhosa* (mice), *Shigella gallinarum* (chick embryo), *Sh. moniliformis* (mice), *Leishmania donovani* (chick embryos), *Staph. aureus* (mice), *Streptococcus pyogenes* (mice), *D. pneumoniae* (mice), and *B. anthracis* (mice). Moderate activity on *H. pertussis*, *Brucella* (mice and guinea pigs), *E. coli* (rabbits), *Proteus* (chick embryo), *Pseudomonas* (mice), *Erysipelothrix rhusiopathiae* (turkeys), *Borrelia novyi* (mice), *Leptospira icterohaemorrhagiae* (Syrian hamsters and rabbits) and other spirochetes, *Clostridium perfringens* but not other clostridia (25). Active on pleuropneumonia-like organisms (rats, chick embryos). Very slightly active on *Rickettsia burneti*, *R. akari*, *R. prowazekii*, *R. rickettsii*, and *R. mooseri*, when given before infection (25). Rickettsiostatic activity in eggs is reversed by X-ray irradiation

(64). Slightly active on *Plasmodium gallinaceum* (chick and tissue culture), but not on *P. cathemerium* or *P. lophurae*. Very high doses increase survival time of mice infected with *P. berghei* (61). Not active on *Trypanosoma* or *Trichomonas*. Accelerates phagocytosis of both resistant and sensitive strains of *E. coli* and *Ps. aeruginosa* in guinea pigs (50). At certain concentrations given intraperitoneally to mice infected with *Sal. typhosa*, increases fatality rate over nontreated controls. At higher concentrations, mice are protected (8).

Animal Nutrition

(See chlortetracycline for a discussion of growth stimulation by antibiotics.) There are many conflicting reports on the role of streptomycin in the nutrition of animals. Some of the positive findings when streptomycin was included in the diet include: *increased growth rate of:* chicks (6), goslings (46), turkey poults (34), weanling pigs (70), dairy calves (84), and silkworms (43), and *increased:* weight of rats (32), egg production and hatchability in pullets (45), and silk production in silkworms (43).

Plant Disease Control

Streptomycin in combination with oxytetracycline is used commercially to control certain plant diseases, such as fire blight. Streptomycin alone or streptomycin-oxytetracycline mixtures have been reported to give various degrees of control of the following diseases, under either greenhouse or field conditions. *Blights:* Halo blight (*Pseudomonas medicaginis* var. *phaseolicola*) of beans (47, 68), common blight (*Xanthomonas phaseoli*) of beans (47), fire blight (*Erwinia amylovora*) of apple (67) and pear (102), bacterial blight (*Pseudomonas apii*) of celery (88), bacterial blight (*Pseudomonas glycinea*) of soybean (122), and walnut blight (*Xanthomonas juglandis*) (102). *Spots:* Bacterial spot (*Xanthomonas vesicatoria*) of tomato and pepper (79), lima bean (128), bacterial leaf spot (*Pseudomonas sesame*)of sesame (97), and angular leaf spot (*Pseudomonas lachrymans*) (90). *Rots:* Bacterial stem and leaf rot (*Erwinia* sp.) of philodendron (92), ring rot (*Corynebacterium sepedonicum*) of potato seed-pieces (29), bacterial rot (*Erwinia atroseptica* and *E. carotovora*) of potato seed-pieces (120), and soft rot (*Erwinia* sp.) of lettuce (87). *Wilts:* Bacterial wilt (*Erwinia chrysanthemi*) of chrysanthemum (71), tomato, alfalfa (102), and cucumber (*Erwinia tracheiphila*) (105). *Cankers, galls, and tumors:* Crown-gall of carrot (21), cherry (101), rose (121) (*Agrobacterium tumefaciens*) and oleander galls (*Pseudomonas tonelliana*) (29), bacterial canker (*Pseudomonas mors-prunorum*) of cherry (108), bacterial stem canker (*Pseudomonas* sp.) of young plum trees (127), and citrus canker (*Xanthomonas citri*) (133). *Miscellaneous:* Blackleg disease (*Erwinia atroseptica*) of potatoes (86), wildfire (*Pseudomonas tabaci*) (80) of tobacco, blast (*Pseudomonas syringae*) of peach (124) and pear (91), infections (*Xanthomonas pruni*) of plum budwood and peach trees (29, 104), infections (*Erwinia carnegieana*) of giant cactus (29), Stewart's disease (*Bacterium stewartii*) of corn (102), silvering disease (*Corynebacterium betae*) of beet (106), and infections (*Xanthomonas translucens*) of wheat seedlings (107). *Fungal diseases:* Despite the fact that streptomycin has no activity *in vitro* against fungi, the following diseases were reported to have been affected to various degrees: loose smut (*Ustilago nuda*) of barley (33), downy mildew (*Phytophthora phaseoli*) of lima beans (98), cucurbits (*Pseudoperonospora cubensis*) (89), hops (123), broccoli (*Peronospora parasitica*) (100), blue mold of tobacco (*Peronospora tabacina*) (78), anthracnose (*Glomerella lindemuthianum*) of beans (81), sugar beet root rot (*Aphanomyces cochlioides* and other fungi) (20), late blight of tomato (*Phytophthora infestans*) (99), and infections of potato stems and tomato plants (*Verticillium* and *Rhizoctonia*) (69).

Miscellaneous

Streptomycin increases mosquito susceptibility to *Plasmodium gallinaceum* infections when given in the diet (49); has a vitamin-sparing action on the virus-tumor tissue of *Rumex acetosa* (62), stimulating it at 2 ppm or below, inhibiting it at higher concentrations (27); prevents soft rot in packaged spinach (66); and prevents fish spoilage (28).

Toxicity: Calcium chloride complex: LD_{50} (mice) 200 mg of base per kg intravenously, >700 mg per kg subcutaneously, and 9000 mg per kg orally. LD_{50} (rats) 175 mg of base per kg intravenously, >600 mg per kg subcutaneously, and >6000 mg per kg orally. LD_{50} (rabbits) 225 mg of base per kg intravenously, and >600 mg per kg subcutaneously. LD_{50} (cats) 150 mg of base per kg intravenously, 600 mg per kg subcutaneously, and >2000 mg per kg orally. Frogs are considered least sensitive to streptomycin-$CaCl_2$; monkeys and dogs most sensitive. *Trihydrochloride:* LD_{50} (mice) 1440 ± 116 mg per kg subcutaneously (13). Poorly absorbed from the gastrointestinal tract.

Search for Salts of Lower Toxicity

Streptomycin and streptidine are toxic to the eighth cranial nerve, giving rise to vestibular dysfunction. Large or prolonged doses produce deafness. Interference with renal function is common, but serious disability is rare except when there is pre-existing kidney disease. Allergy to streptomycin (dermatitis, etc.) occurs in certain patients, and in persons such as nurses and agricultural workers who are in rather constant contact with the drug (25). Calcium was the first substance reported to have a detoxifying effect on streptomycin (26). German workers (83, 95) reported that use of streptomycin pantothenate, instead of the sulfate, reduced acute and vestibular toxicity in animals. Subsequently, they found that this reduction of toxicity was partly caused by the presence of calcium in their preparations (from the calcium pantothenate that was used). Nevertheless, they maintained that streptomycin pantothenate containing less than 1 per cent calcium was still considerably less toxic than streptomycin sulfate (96, 114). *Sulfate:* LD_{50} (mice) 640 mg (as base) per kg subcutaneously. *Pantothenate:* LD_{50} (mice) 1050 mg (as base) per kg, subcutaneously (96). Certain workers subsequently confirmed these findings (115, 116); others differed (117). In some reports (115, 116), the tripantothenate was found to be somewhat less toxic than the sulfate, but not to the degree reported by the German workers. Furthermore, the tripantothenate was more toxic than the $CaCl_2$ complex. No reduction in vestibular toxicity was reported by either group but a reduced ototoxicity to rats was reported (116). LD_{50} values reported are as follows (115): *Sulfate:* LD_{50} (mice) 103.5 mg per kg intravenously. *Tripantothenate:* LD_{50} (mice) 140 mg per kg intravenously. *$CaCl_2$ complex:* LD_{50} (mice) 169 mg per kg intravenously. The pantothenates are not used clinically in the United States.

Fish, Mollusks, Nematodes

Streptomycin produces neurological symptoms in goldfish, when it is dissolved in tank water (112). Toxic to the snail, *Australorbis glabratus*, inducing behavioral abnormalities and inhibition of growth and reproduction. This action is reversed by Ca^{++}, but not Mn^{++} (134). Slightly inhibitory to the nematode *Rhabditis briggsae* (77).

Plants, Algae, Protozoa, Fungi

Exposure to certain concentrations of streptomycin may obliterate chloroplasts in seed plants (16) and in certain algal flagellates (38). In plants, chlorosis, or lack of chlorophyll, is not always permanent (102), but sensitive plants may remain chlorotic and stunted after exposure to sufficiently high concentrations (103). In certain algae, such as *Euglena gracilis*, bleached strains produced by exposure to streptomycin may be maintained indefinitely on simple synthetic media (38). Carotenoid synthesis is reduced also, but not as much as chlorophyll synthesis (110). These bleached cells can survive 5 mg per ml of streptomycin, whereas other genera are susceptible to 1 to 5 μg per ml. On the other hand, *Chlorogonium euchlorum* is bleached by streptomycin, but no colorless strain can be propagated. Resistance to bleaching and killing can be induced in certain algae; however, chloroplasts may be abnormal, and the pyrenoid much reduced. Leucoplasts are unaffected. Resistance to killing can also be induced in certain normally colorless protozoa. In bleached stains, the stigma is reduced in size and the effect on the eye spot is variable (38). Dihydrostreptomycin is also active, but streptobiosamine, streptamine, and N-acetylglucosamine are inactive (42). Plants such as barley, germinating on a streptomycin-containing medium, produce white coleoptiles and first leaves containing neither chlorophyll nor carotenoids, but normal amounts of anthocyanins. Catalase activity is depressed (16). In cabbage seedlings, a purple discoloration and death follow seed treatment with 25 ppm. At 480 ppm, applied as a spray, transient chlorosis in tobacco plants has been noted (80). Na-K chlorophyllin added to a 1000-ppm spray of streptomycin was reported to have prevented achlorosis in bean and cucumber plants (129). In the fungus *Phycomyces blakesleeanus*, carotenoid synthesis, but not growth, is inhibited by 0.1 to 0.2 per cent (weight per volume) streptomycin or streptobiosamine in an asparagine-glucose medium. Replacement of the asparagine in whole or in part with an ammonium salt permits carotenoid synthesis to occur. If acetate replaces the glucose, both growth and carotenogenesis are inhibited (110).

Effect on Growth

Inhibition: Stem and root growth are inhibited when tomato seedlings are grown in a nutrient solution containing 25 ppm of streptomycin sulfate. Mn^{++} and, to a lesser extent, Ca^{++}, reverse this effect. As a spray, 10,000 ppm causes temporary stunting and spotty apochlorosis of the stems, followed by growth greater than the control; root growth is also stimulated. Streptidine and streptobiosamine show no effect (94). Root growth (percentage of control) is inhibited at 100 ppm in

nutrient solution: mustard (80 per cent), red clover (61 per cent), cucumber (54 per cent), and wheat (46 per cent) (85). Celery and radish plants are most sensitive, lettuce moderately sensitive, and pea, pepper, and watermelon tolerant to concentrations of 1:25 (103). Not toxic to cucumber, tomato, barley, safflower, or sunflower seeds soaked for 24 hours in 200 μg per ml (12), or to wheat, white mustard, or red clover seeds soaked in 25 ppm (35). Nutrient solutions containing 50 ppm are inhibitory to growth, but sprays of 200 ppm do not affect growth or alkaloid content of tobacco plants (109). *Stimulation:* Wheat root growth is enhanced when grown in water containing low concentrations of streptomycin (48). Fresh weight of tobacco plants grown in a nutrient solution containing 10 ppm of streptomycin is greater than the control (109). Stimulates the virus-tumor tissue of *Rumex acetosa* at 2 ppm or below (27).

Cells

Least injurious doses for spleen and skin cells in tissue culture are 155 to 310 and 210 to 415 μg per ml, respectively (75). Highest concentration permitting epithelial cell migration in tissue culture is 3.6 mg per ml (131). Minimal concentration causing degeneration of HeLa cells is 10 to 20 mg per ml (119). Toxic to *Allium cepa* root cells at >100 ppm (30).

Utilization: Tuberculosis. Infections caused by gram-positive and gram-negative bacteria, including bacteremia, meningitis, tularemia, brucellosis, plague, and others (25).

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Streptonigrin

Produced by: Streptomyces flocculus.

Method of extraction: Extraction of the filtered broth with n-butanol or ethyl acetate between pH 3.0 and 5.0. Extract concentrated. Back-extracted into a neutral buffer. Re-extraction into ethyl acetate at pH 4.0. Concentration of the solvent extract yields a coffee-brown powder. Countercurrent distribution (100 transfers) in the system ethyl acetate-3 per cent phosphate buffer at pH 7.5. The distribution is followed by measurements of optical density at 370 mμ and biological activity against *Staph. aureus*. Coefficient distribution of streptonigrin varies with concentration.

Chemical and physical properties: Coffee-brown to almost black, long, rectangular plates. Decomposes at 275°C. Slightly soluble in water, lower alcohols, ethyl acetate, and chloroform. More soluble in dioxane, pyridine, dimethylformamide, and aqueous sodium bicarbonate. Bicarbonate solutions decompose slowly. Tentative empirical for-

mulas: $C_{24}H_{20}O_8N_4$ or $C_{24}H_{22}O_8N_4$. Weak acid. Infrared absorption spectrum given in reference 1. Maxima of light absorption in methanol at about 380 and 245 mμ. Stable at room temperature for 24 hours and at 100°C for 15 minutes between pH 2.0 and 7.0. Above pH 8.0, decomposition occurs. Photosensitive at pH 7.0 and higher. Dark greenish brown alcoholic $FeCl_3$ test. Deep yellow color in concentrated sulfuric acid. In aqueous sodium hydroxide, the solutions are dark greenish brown, changing to red with evolution of ammonia. Orange-red precipitate with 2,4-dinitrophenylhydrazine.

Biological activity: Active against gram-positive and gram-negative bacteria. Very active against adenocarcinoma 755 in mice and against HS No. 1 in rats. Moderately active against sarcoma 180 and leukemia 1210.

Reference: 1. Rao, K. V. and Cullen, W. P. Antibiotics Ann. 1959–1960, pp. 950–953.

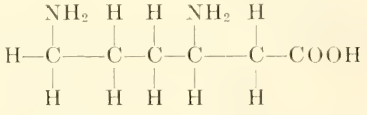
Streptothricin

Produced by: Streptomyces lavendulae strains (1, 3, 15), *Streptomyces* sp. (15, 17, 18), *Streptomyces griseus* f. *farinosus* (32). The culture described in reference 17 may also produce streptomycin; the one described in reference 18 may also produce streptolin.

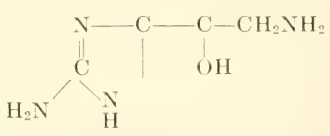
Synonym: See streptothricin-like antibiotics.

Method of extraction: Adsorbed from broth-filtrate on activated charcoal at neutral pH. Eluted with dilute mineral acid (A), acidified ethanol (B), or formic acid in aqueous methanol (C). Eluates A or B neutralized, then concentrated *in vacuo* at 50°C. Residue taken up in absolute ethanol, filtered, evaporated, then taken up in water (1, 3, 13). Eluate C concentrated *in vacuo*; methanol and acetone added in excess to precipitate crude streptothricin (13). Precipitated as phosphotungstate or picrate, then purified by salt conversion (11, 13, 20, 23). Fractional crystallization of a pure reineckate (11) or crystalline helianthate (12). Purification by chromatography on alumina (20) or a Darco G-60-filter paper pulp mixture, with methanol as solvent and developer in both instances (13). Can also be chromatographed on an activated-carbon column from a neutral aqueous solution and eluted with very dilute acetone (1 per cent; volume per volume) (27, 34).

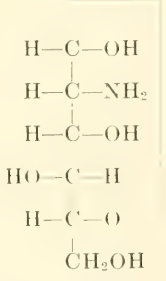
Chemical and physical properties: Tribasic substance (27). *Sulfate:* Amorphous white powder. Decomposes at 213–217°C. Soluble in water and dilute mineral acids. Destroyed by strong acid. Insoluble in ether, petroleum ether, and chloroform (1). Ultraviolet absorption spectrum shows only end-absorption (11). Positive Benedict, Tollen, neutral $KMnO_4$, Fehling (boiling), biuret, ninhydrin, Pauly, Elson-Morgan, and anthrone (weak) tests. Negative Hopkins-Cole, Millon, $FeCl_3$, nitroprusside, Molisch, Sakaguchi, and Schiff tests (11, 27). pK_a values of 7.1, 8.2, and 10.1 (27). Van Slyke N = 20 to 25%. No O—CH_3, N—CH_3, or acetyl groups (11, 27). *Hydrochloride:* White powder. $[\alpha]_D^{25} = -49°$ (c = 1 per cent in water) (13, 20). $C_{20}H_{34}N_8O_9 \cdot 3HCl$: C = 38.22%; H = 5.79%; N = 17.79%; Cl = 16.34% (27). Thermostable. More stable to acid than to alkali (11). *Reineckate:* Clusters of fine platelets. Sinters at 184°C; decomposes at 192–194°C. $C_{21}H_{39}O_3N_{17}S_8Cr_2$ (base: $C_{13}H_{25}O_7N_5$): C = 24.95%; H = 4.12%; N = 24.0%; Cr = 10.32%; S = 25.8% (11). *Helianthate:* m.p. 220–225°C (decomposition) (12), or 225–230°C (13). Streptothricin also forms crystalline complex salts with calcium chloride and other alkaline earth metal halides. *Hydrochloride-$CaCl_2$ complex:* m.p. about 225°C (decomposition). $[\alpha]_D^{25} = -46.5°$ (c = 0.95 per cent in water) (20). Streptothricin cannot be hydrogenated (27). Acid hydrolysis products include: L-β-lysine, streptolidine, and 2-amino-2-desoxy-α,D-gulose.



Scheme 11. L-β-Lysine (β,ε-diamino-n-caproic-acid).



Scheme 12. Streptolidine (roseonine, geamine): 2-amino-4-(1-carboxy-1-hydroxy-2-amino)ethyl-2-imidazoline.



Scheme 13. 2-Amino-2-desoxy-α-D-gulose (α-D-gulosamine).

Other products are described in reference 29. (See Table 43.)

Biological activity: In vitro: Active on gram-positive and gram-negative bacteria, including mycobacteria (5). Active on certain fungi and yeasts (6). Slightly active on *Trichomonas vaginalis* (25 μg per ml) (26). Active on coliphage (8). Resistance to streptothricin developed less readily than to streptomycin in most bacteria tested (16). Active in mice on *Streptococcus hemolyticus, Sal. schottmülleri, Sal. typhimurium, E. coli, Shigella dysenteriae,* and *Pasteurella tularensis.* Most gram-positive bacteria are resistant to streptothricin *in vivo,* as are *Pr. vulgaris, Ps. aeruginosa,* influenza virus, and *Trypanosoma equiperdum* (4, 7). Active in chicken embryos and guinea pigs against *Br. abortus* but not *M. tuberculosis* (2, 9). Active in rats and dogs on *Endamoeba histolytica* (31). Prolongs survival time in mice with *Cryptococcus neoformans* infections (19), and is active against *Blastomyces dermatitidis* infections in the chick embryo, although irritating to the embryo (14). Cross-resistance with streptolin, neomycin, and streptomycin. Increases growth rate of chickens on a vegetable protein diet (24). Actively translocated by tobacco, tomato, and bean plants (28). Prolongs survival time in mice infected with *Clostridium perfringens* and *Cl. histolyticum* (33).

Toxicity: Acute intravenous LD_{50} (mice) >245 mg per kg; however, delayed toxicity kills at this level. If this delayed toxicity is considered, mice tolerate only 8 mg per kg (10). Toxic to *Allium cepa* root cells at 50 ppm (22).

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Streptothricin-like Antibiotics

Produced by: Streptomyces sp. resembling *S. roseochromogenes* (4, 5, 7, 8, 10), *S. flavochromogenes* (12), *Streptomyces* sp. resembling *S. flavus* (14), The last culture also produces flavacid.

Synonyms: Antibiotic 20, antibiotic 24, antibiotic 39, antibiotic 259, antibiotic 120 (Seki substance), roseocitrin A (12), and streptothricin III (14). See Table 43.

TABLE 43

Rf values of streptothricin and related antibiotics (Horowitz and Schaffner, 1958)

Circular paper chromatography (1-propanol-pyridine-acetic acid-water, 15:10:3:12).

Antibiotic	Minimal No. of components	Rf value of salts	
		-HCl	$-SO_4$
Streptothricin	1	0.50	0.43
Streptothricin VI	2	0.35, 0.50	0.32, 0.43
Pleocidin complex	4	0.22, 0.35, 0.42, 0.50	0.20, 0.32, 0.37, 0.43
Viomycin	1	0.42	0.38
Antibiotic 136	3	0.35, 0.40, 0.50	0.31, 0.43
Streptolin A	2	0.24, 0.33	0.20, 0.33
Streptolin B	3	0.30, 0.35, 0.50	0.27, 0.32, 0.43
Antibiotic VIIa (from streptolin broth)	3	0.30, 0.33, 0.44	0.27, 0.31, 0.41
Antibiotic IXa (from streptolin broth)	2	0.30, 0.44	0.27, 0.41
Mycothricin complex	4	0.26, 0.35, 0.42, 0.50	0.23, 0.32, 0.37, 0.43
Geomycin	2	0.40, 0.33 (diffuse bands)	0.35, 0.26 (diffuse bands)
Roseothricin A	2	0.30, 0.50	0.32, 0.43
Roseothricin B	1	0.31	0.27
Roseothricin C	1	0.28	0.24

Remarks: Since Waksman and Woodruff (1) described streptothricin in 1942, investigators all over the world have described many antibiotics which have been termed "streptothricin-like" substances. Whether many of these have been reported because there are comparatively large numbers of actinomycetes that produce them in soil, or because these substances are relatively easy to produce and isolate is not clear. Nevertheless, despite the fact that streptothricin itself has never been completely characterized chemically, many basic water-soluble substances have been described as members of this group. As a rule, such substances have the following characteristics in common: they form amorphous hydrochlorides and sulfates, crystalline helianthates and/or reineckates, are insoluble in most organic solvents, and show end-absorption in ultraviolet light. Many have been further characterized by their reactions to qualitative organic tests, but except for negative maltol and Sakaguchi reactions (differentiating them from streptomycin) their reactions to a given test vary. Certain of them have hydrolysis products in common with each other and with streptothricin. Racemomycin B, streptolin, roseothricin, geomycin, and streptothricin all yield β-lysine and 2-amino-4 (1-carboxy - 1 - hydroxy - 2 - amino)ethyl - 2 - imidazoline ("streptolidine," "roseonine," or "geamine") on hydrolysis (19, 20, 23). These may also be products of mycothricin hydrolysis (21). Many are complexes containing various closely related components. Horowitz and Schaffner (22) have shown by paper chromatography that some of the streptothricin-like substances have components in common (Table 43). Other workers have reported that actinorubin, lavendulin, roseothricin, antibiotic 136, and streptothricin VI contain streptothricin and two or more components of streptolin (2, 3). Variation in the amounts of components in each of these complexes undoubtedly accounts for the difficulties that have been encountered in characterizing and purifying them, and for the variations in biological activity which have been reported. Japanese workers have been especially active in reporting such substances. They have attempted to bring order out of chaos by dividing them into two groups. The first (streptothricin II type) contains the highly toxic, highly active type which diffuses slowly in agar. This group would contain streptothricin. The second (streptothricin I type) contains those which are relatively nontoxic, less biologically active, and diffuse readily in agar. This group includes roseomycin. Like all generalizations, this one has exceptions. A description of some of these antibiotics follows. For others, see actinorubin, antibiotic 136, antibiotic 156, antibiotic EI_5, antibiotic of Mukherjee, fuscomycin,

grasseriomycin, geomycin, lavendulin, mycothricin, novomycin, pleocidin, racemomycin, streptin, streptolin, roseocitrin B, and viomycin, as well as others less well described.

Method of extraction: Essentially like that for streptothricin (10, 14).

Chemical and physical properties: All substances above except streptothricin III: Colorless basic substances. Water-soluble. Helianthates and reineckates precipitate as platelets. Helianthates are soluble in 50 to 100 per cent methanol; slightly soluble in ethanol; scarcely soluble in propyl, isopropyl, butyl, and amyl alcohols, chloroform, and ether. Hydrochlorides give positive Molisch, Tollen, Fehling, indole, and glucosamine tests; negative maltol, biuret, ninhydrin, xanthoproteic, Adamkiewitz, Liebermann, Neubauer, and Sakaguchi tests (4). *Streptothricin III:* Positive Molisch and glucosamine tests. Negative Sakaguchi, maltol, Fehling, $FeCl_3$, ninhydrin, biuret, Millon, and Hopkins-Cole tests (14). Melting points of the helianthates and reineckates have been reported as follows (the first figure is for the helianthate, the second for the reineckate): antibiotic 24: 275–285°C, 185–210°C; antibiotic 39: 225–230°C, 198–201°C; antibiotic 259: 244°C, 193–196°C; antibiotic 120 (Seki): 224–230°C, 184–185°C; roseocitrin A: 223–225°C (no report for the reineckate); streptothricin III: 208–211°C (decomposition), 172–178°C (decomposition).

Biological activity: All have activity which is qualitatively like that of streptothricin, with small variations. Antibiotics 20, 24, 39, and streptothricin III have relatively low activity on *Staph. aureus*. Antibiotics 259 and 120 are more active. Antibiotic 39 diffuses readily in agar, 24 and 259 to a lesser degree, and 120 and 20 do not diffuse readily. Cross-resistance with each other and partial cross-resistance with streptomycin (4, 14). *In vivo: Antibiotic 20:* Active on *Pasteurella tularensis* in mice (13). *Antibiotic 259:* Active on *Sal. typhosa* in mice (15). *Antibiotic 120:* Inactive in mice on *Hemophilus pertussis* (6). *Streptothricin III:* Active on influenza PR 8 virus (tissue culture of chick embryo chorioallantoic cells), but has no virucidal effect and does not prevent adsorption on the host cell. Viral development affected if antibiotic added as late as 10 hours postinfection (14).

Toxicity: Antibiotic 20: Reputed to have "low" toxicity, of the order of that of roseomycin (6). *Antibiotic 24:* A single intravenous dose of 5 mg per mouse is lethal in 12 days (4), but 20 mg per mouse, intramuscularly, is tolerated. *Antibiotic 39:* A single intravenous dose of 5 mg per mouse is lethal in 6 days (4). *Antibiotic 259:* A single intravenous dose of 1.5 mg per mouse is lethal in 6 days (4). *Antibiotic 120:* A single intravenous dose of 10 mg per mouse is tolerated (4). *Streptothricin III:* A single intravenous dose of 0.63 mg per mouse is nontoxic; 2.5 mg per mouse is lethal in 4 days (14).

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Streptovaricins

Produced by: Streptomyces spectabilis.

Synonym: Dalacin.

Method of extraction: Culture-filtrate extracted with ethyl acetate. Concentrated extract precipitated by addition of 4 volumes of technical hexane. Precipitate dissolved in methyl chloride and reprecipitated by addition of 5 volumes of hexane. Partially crystalline precipitate washed with hexane and dried *in vacuo* to give the streptovaricin "complex" (2). Components separated by chromatography (agent not stated, toluene-ethylene glycol system) (7), or by countercurrent distribution (water-95 per cent ethanol-cyclohexane-ethyl acetate, equal parts) (2).

Chemical and physical properties: "Complex" is said to be made up of 60 per cent antimicrobial substances and 40 per cent inactive material. Partially crystalline and bright orange-yellow material; m.p. 144–147°C (decomposition). Data given for a specific lot of the "complex." Soluble in dimethylformamide and 95 per cent ethanol in excess of 500 mg per ml. Soluble in methanol, butanol, lower ketones, methylene chloride, chloroform, and the lower acetates. Slightly soluble in water (0.7 mg per ml). Nearly insoluble in hexane, ether, carbon tetrachloride, and benzene. Specific absorbance of 7.7 at 435 mμ (visible light) is destroyed in alkaline but not acidic solution; ultraviolet absorption maximum at 244.5 mμ, with a shoulder at 263 mμ and a plateau at 316 mμ. $[\alpha]_D^{24} = +288°$ (c = 0.31 per cent in absolute ethanol). Infrared spectrum given in reference 2. Indicator properties: yellow at acid pH; red-amber at alkaline pH. Positive $FeCl_3$ and iodoform tests. Negative Benedict, ninhydrin, Molisch, Zimmerman, and Liebermann tests. Labile to alkali; practically all activity irreversibly lost in 3 days at pH 7.8. Complex and Components A, B, and C quite unstable in distilled water, pH 6.8 buffer, or organic culture medium at 37°C, losing 85 to 90 per cent of activity in 40 hours. Components A to E are bright orange substances having the following Rf values on paper chromatography: 0.13, 0.37, 0.77, and 0.88; the last two do not separate in this system (cyclohexane-chloroform-water, 1:8:2) (1, 2, 5, 7, 9).

Biological activity: Complex: Active on gram-positive and some gram-negative bacteria and fungi. Active on *M. tuberculosis* H37Rv and BCG at 0.16 μg per ml. No cross-resistance between streptovaricin and the major antibiotics is observed in micrococci. Development of resistance follows two patterns, one fast, the other slow, depending on the component and the organism used. No cross-resistance between components. Components A and B more active than C on *M. ranae*, but all are equally active against *B. subtilis*. A and B also active on certain gram-negative bacteria and fungi. D and E have no activity on gram-negative bacteria and only slight activity on gram-positive bacteria and mycobacteria. *In vivo:* Complex is active against *M. tuberculosis* var. *hominis* H37Rv and *M. tuberculosis* var. *bovis* (Vallee) in mice and guinea pigs. Streptovaricin-sensitive, virulent, acid-fast bacilli can be isolated from tissues of streptovaricin-treated, surviving mice that show no gross lesions. In mycobacteria, development of resistance to streptovaricin is accompanied by reduction of virulence to mice. Active on murine leprosy. Component A has no antimycobacterial activity *in vivo*; C has more than the complex (1, 3–5, 8).

Toxicity: Mice tolerate 1000 mg per kg per day by oral intubation, and 800 mg per kg per day subcutaneously for 6 days (3).

References:

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2. Whitfield, G. B. *et al.* Am. Rev. Tuberc. **75:** 584–587, 1957.
3. Rhuland, L. E. *et al.* Am. Rev. Tuberc. **75:** 588–593, 1957.
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6. Nathan, A. Trans. 17th Veterans Admin. Conf. Chemotherapy Tuberc., pp. 336–338, 1958.
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Streptovitacins

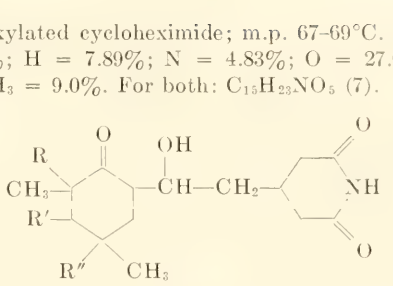
Produced by: Streptomyces griseus. This culture also produces cycloheximide.

Remarks: Streptovitacins A and B are isomeric hydroxylated cycloheximide derivatives (3).

Method of extraction: Whole culture adjusted to pH 3.5, heated to 60°C, cooled, and filtered. Cycloheximide and other constituents removed by extraction of filtrate with chloroform or methylene chloride. Streptovitacins adsorbed on carbon and eluted with acidic aqueous 85 per cent acetone. Eluate concentrated by vacuum distillation. Residue adsorbed on Permutit DR from aqueous solu-

tion, and desorbed by gradient elution with water to 50 per cent ethanol. Active fractions concentrated, then chromatographed on Dicalite (lower phase of an ethyl acetate-cyclohexane-pH 5.0 McIlvaine's buffer (7:1:8) as solvent and the upper phase as developer). Active fractions containing streptovitacin A are azeotropically distilled and freeze dried. A can be crystallized from acetonitrile. Recrystallized from acetonitrile, *n*-butanol, ether, and ethyl acetate. Can also be purified by countercurrent distribution (n-amyl alcohol-isoamyl alcohol-water, 12:17:29) (2). B can be crystallized from *n*-butanol-cyclohexane, and recrystallized from acetonitrile-ether.

Chemical and physical properties: Complex with five components, two major isomeric ones, A and B, and three minor, C, D, E (1). Neutral substances. *Streptovitacin A:* Exists in two crystalline modifications (crystallographic data given in reference 2). Orthorhombic form shows transformation to the monoclinic form at 147°C. Monoclinic form melts at 156–161°C. Soluble in water; less soluble in polar organic solvents; essentially insoluble in nonpolar solvents. Only end-adsorption in ultraviolet light. Infrared spectrum given in reference 2. Little rotation at the sodium D line; optical activity in dioxane shown by rotary dispersion curve in reference 2. Stable at 70°C for 60 days in dry form. Relatively stable in aqueous solution at acidic but not alkaline pH (2). Rf = 0.38 on paper chromatography (water-saturated ethyl acetate, paper impregnated with 0.1 *M* phosphate solution at pH 4.0) (1). $C_{15}H_{23}NO_5$: C = 60.71%; H = 8.05%; N = 4.73%; O = 27.00%; C—CH_3 = 7.7% (2). No titratable groups. Alkaline degradation products include ammonia, a small amount of 2,4-dimethyl-2-cyclohexanone, and hydroxydimethylcyclohexanone. A is 3-[2-(5-hydroxy - 3,5 - dimethyl - 2 - oxocyclohexyl) - 2 - hydroxyethyl] glutarimide (see structural formula below). *Monoacetate:* m.p. 165–168°C (7). *Streptovitacin B:* Crystalline substance; m.p. 124–128°C. Solubilities probably the same as A. Only end-absorption of ultraviolet light. Infrared spectrum and optical activity given in reference 2. Rf = 0.45 (same system as for A). $C_{15}H_{23}NO_5$: C = 60.66%; H = 7.87%; N = 4.71%; O = 27.57%; C—CH_3 = 8.6% (2). B is 3-[2-(4-hydroxy-3,5-dimethyl - 2 - oxocyclohexyl) - 2 - hydroxyethyl]-glutarimide (see structural formula below). *Diacetate:* m.p. 155–158°C (7). *Streptovitacin C_2 and streptovitacin D:* C_2 : m.p. 91–96°C. C = 60.53%; H = 7.98%; N = 4.67%; O = 27.71%; C—CH_3 = 8.2%. C_2 is 3-[2-(3-hydroxy-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutarimide. D: Ring-hydroxylated cycloheximide; m.p. 67–69°C. C = 60.42%; H = 7.89%; N = 4.83%; O = 27.00%; C—CH_3 = 9.0%. For both: $C_{15}H_{23}NO_5$ (7).



Streptovitacin A: R = R′ = H; R″ = OH.

Streptovitacin B: R = R″ = H; R′ = OH.

Streptovitacin C: R′ = R″ = H; R = OH (7).

Biological activity: Streptovitacins A and B: Active on *Sacch. pastorianus* at 5 μg per ml. Active on *Trichomonas vaginalis* (1). *In vivo:* Active, if given 24 hours after implantation, on sarcoma 180 (ascites), Ehrlich ascites carcinoma (some effect on solid forms at high doses) in mice, Walker adenocarcinoma (rat), Guérin adenocarcinoma (rat), Jensen sarcoma (in rats), RC carcinoma, and leukemia L-4946 (mice). Not effective on the Murphy-Sturm lymphosarcoma. Less effective on established tumors. Affects the ability of sarcoma 180 to regenerate (4, 5). Active on Eagles's KB epidermoid carcinoma in tissue culture (6).

Toxicity: Symptoms at high dose levels include listlessness, diarrhea, and hematuria (4).

References:

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3. Herr, R. R. Antibiotics Ann. 1958–1959, pp. 560–564.
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5. Field, J. B. *et al.* Antibiotics Ann. 1958–1959, pp. 572–579.
6. Smith, C. G. Proc. Soc. Exptl. Biol. Med. **100:** 757–759, 1959.
7. Herr, R. R. J. Am. Chem. Soc. **81:** 2595–2596, 1959.

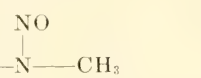
Streptozotocin

Produced by: Streptomyces achromogenes var. *128* (1).

Method of extraction: Filtration of broth at pH 4.0. Broth concentrated *in vacuo* to 0.08 volume and added to 5 volumes of acetone. Precipitate discarded. Acetone concentrated to an aqueous solution and freeze dried. Chromatography on

Dicalite column with solvent system isobutanol-cyclohexane-pH 4.0 buffer (20:4:4). The products of the peak fractions again chromatographed on Dicalite, with the solvent system methyl ethyl ketone-cyclohexane-pH 4.0 buffer (9:1:1.43). Active fractions pooled and distributed for 775 transfers between methyl ethyl ketone and water. Active fractions pooled and water removed by distillation. Crystallization from anhydrous methyl ethyl ketone solution. Recrystallization from 95 per cent ethanol (2).

Chemical and physical properties: Crystalline product is a mixture of crystals of various types. Very soluble in water; soluble in lower alcohols; relatively insoluble in less polar solvents. Decomposes with evolution of gas at about 115°C and becomes a clear liquid by 125°C. Tentative empirical formula: $C_{14}H_{27}N_3O_{12}$. Maximal absorption of ethanolic solution at 228 mμ, with very weak maxima at 380, 394, and 412 mμ. Infrared absorption data given in reference 1. Maximal stability at pH 4.0. No loss of activity of dry samples at room temperature for 30 days and at 4°C for 6 months. In 10 per cent aqueous sodium hydroxide, streptozotocin decomposes immediately, with production of the gas diazomethane. Positive Liebermann nitroso test. Negative Benedict, ninhydrin, and biuret tests. After evolution of diazomethane, acidification causes evolution of carbon dioxide. Oxidation of residue with periodate gives aldehyde and formic acid. The following N-nitrosomethylamide function is suggested to exist in streptozotocin (2):



Biological activity: Active against gram-positive and gram-negative bacteria. Little or no action against strains of *Ps. aeruginosa*, *Sh. dysenteriae*, *K. pneumoniae*, *Neisseria*, *Clostridium*, and *Hemophilus*. Bacteria were incubated for only 8 hours before end-points of activity were determined because the antibiotic is not stable in most laboratory media over a 24-hour period. No bactericidal activity (3). Development of resistance rapid. No cross-resistance with commonly used antibiotics (4). Active in mice infected with *Staph. aureus*, *Pasteurella multocida*, and *Pr. vulgaris* when administered subcutaneously or orally (3).

Toxicity: Mice tolerate 200 mg per kg per day for 14 days orally.

References:

1. Vavra, J. J. *et al.* Antibiotics Ann. 1959–1960, pp. 230–235.
2. Herr, R. R. *et al.* Antibiotics Ann. 1959–1960, pp. 236–240.
3. Lewis, C. and Barbiers, A. R. Antibiotics Ann. 1959–1960, pp. 247–254.
4. Hanka, L. J. and Sokolski, W. T. Antibiotics Ann. 1959–1960, pp. 255–261.

Sulfactin

Produced by: Streptomyces sp. resembling *S. roseus* (1).

Method of extraction: Broth-filtrate adjusted to pH 7.0 with H_2SO_4, and extracted with n-butanol. Extract evaporated *in vacuo* at 45°C. Residue treated with boiling ether, then extracted with chloroform. Purification by chromatography on Florisil from chloroform, and elution with 10 per cent ethanol in chloroform. Active fractions evaporated to dryness *in vacuo*. Crystallized from a chloroform solution by concentration and addition of ethanol; or from boiling ethanol (1).

Chemical and physical properties: White needles (from ethanol) or double pyramids (from chloroform); m.p. 245–275°C (decomposition). Very soluble in chloroform; soluble in ethanol, ethyl acetate, dioxane, and butanol; somewhat soluble in methanol. Positive Fehling (on boiling) test. Negative Sakaguchi, Molisch, biuret, $FeCl_3$, and $KMnO_4$ tests. Dialyzes with loss of activity. Not destroyed in boiling ethanol. C = 50.28%; H = 6.04%; N = 17.21%; S = 14.11%. $C_{38}H_{55}N_{11}O_7S_4$ or $C_{27}H_{40}O_5N_8S_3$ (1).

Biological activity: Active on certain gram-positive bacteria, and on *Neisseria sicca* alone of the gram-negative bacteria tested. Not active on mycobacteria. Active on *D. pneumoniae* infections in mice (2).

Toxicity: LD_{50} (mice) 148.5 mg per kg intraperitoneally.

References:

1. Junowicz-Kocholaty, R. *et al.* J. Biol. Chem. **168:** 765–769, 1947.
2. Morton, H. E. Proc. Soc. Exptl. Biol. Med. **66:** 345–348, 1947.

Sulfocidin

Produced by: Streptomyces sp.

Method of extraction: Filtered broth is extracted with chloroform; extract concentrated *in vacuo* and purified by Florisil chromatography (10 per cent ethanol in chloroform, eluant). Active eluates concentrated to dryness. Methanol-extract of residue filtered and concentrated to dryness. Residue extracted with chloroform, and crude sulfocidin precipitated by adding petroleum ether with vigorous stirring. Chromatography on carbon-

Celite, and eluant (chloroform) concentrated to dryness. Residue dissolved in methanol and passed through activated alumina. Most active eluates concentrated to dryness. Residue dissolved in chloroform and treated with petroleum ether to incipient turbidity. Crystals form after 72 hours at 10°C.

Chemical and physical properties: Neutral, yellow-brown crystals; m.p. 166–178°C. C = 64.88%; H = 8.38%; N = 4.25%; S = 1.80%. Ultraviolet absorption peak at 275 to 280 mμ (ethanol). Infrared spectrum given in reference 1. Nitroprusside (for SH groups), azide-iodine (for amino S), ninhydrin, Sakaguchi, maltol, $FeCl_3$, Fehling, biuret, nitrochromic acid, 2,4-dinitrophenylhydrazine, and anhydrous aluminum chloride tests negative. Decolorizes a $KMnO_4$-acetone solution in the cold. Soluble in chloroform, acetone, lower alcohols, 1,4-dioxane, pyridine, glacial acetic acid, and ethyl acetate. Insoluble in water, 10 per cent HCl, 10 per cent $NaHCO_3$, CCl_4, diethyl ether, and petroleum ether. Slightly soluble in 10 per cent NaOH. $[\alpha]_D^{25} = -58.5°$ (c = 0.51 per cent in chloroform). Solutions lose 25 to 50 per cent of their potency in 24 hours in water or buffered solutions (pH 5 to 8) at 25 and 37°C. At 7°C, solutions lose potency slightly during 10 days. Dry powder stable at 7 and 25°C for more than 1 year. Purified but not crystalline product was found to have four fractions, with Rf values of 0.0, 0.06, 0.13, and 0.30 on descending paper chromotography with aqueous 3 per cent NH_4Cl solution (1).

Biological activity: Active on gram-positive and gram-negative bacteria, mycobacteria, and fungi. Strongly bactericidal. Activity unaffected by saliva or serum (1). Has antitumor activity against the ascitic form of Gardner lymphosarcoma and sarcoma 180, but is not active on the solid form of sarcoma 180 or C3HBA adenocarcinoma in mice (2).

Toxicity: LD_{50} (mice) 5 mg per kg intraperitoneally, 10 to 25 mg per kg orally (1), 0.5 to 0.9 mg per kg intravenously (2).

References:

1. Zief, M. *et al.* Antibiotics Ann. 1957–1958, pp. 886–892.
2. Pugh, L. H. *et al.* Antibiotics Ann. 1957–1958, pp. 972–976.

Taitomycin

Produced by: Streptomyces afghaniensis, said to resemble *S. collinus* and *S. erythrochromogenes* (1).

Method of extraction: Present in both mycelium and broth. Minor amount in broth precipitated by adjusting to pH 4.0. All solids from filtration extracted with methanol at 30–35°C with stirring. Extract flash-evaporated, then concentrated *in vacuo*. Aqueous residue extracted with ethyl acetate. Extract evaporated under reduced pressure, residue washed with petroleum ether, and dried. Purified by chromatography from acetone, with 80 per cent methanol as developer. Acidification to pH 4.0 precipitates taitomycin from active fractions. Further purified by countercurrent distribution (pH 8.3 phosphate buffer-methanol-ethyl acetate-chloroform, 1:1:1:1) (1).

Chemical and physical properties: Pale yellowish brown or reddish powder (1). Soluble in pyridine, glacial acetic acid, methanol, ethanol, acetone, ethyl acetate, and alkaline water. Slightly soluble in ether and benzene. Insoluble in petroleum ether, ligroin, and acidic water. Ultraviolet absorption spectrum maxima at 330 and 420 mμ (c = 2 per cent in methanol). Infrared absorption spectrum given in reference 2. Positive Fehling test. Elson-Morgan test becomes positive on mild acid hydrolysis. Questionable biuret and Millon tests. Acid hydrolysates give a positive ninhydrin test. Data on paper chromatography given in reference 2. C = 53.57%; H = 4.87%; N = 9.50%; ash = 2.8%. No data on S, halogen, or Fe content.

Biological activity: Active on gram-positive bacteria and *Neisseria*, but not on *Hemophilus*, *Klebsiella*, or other gram-negative organisms. Not active on fungi, yeasts, or trichomonads. No antitumor activity (2). Not affected by serum, but has hemolytic properties (2). Active in mice on *D. pneumoniae* (2), but not on *Toxoplasma gondii* (3). Active on *Rickettsia mooseri* (chick embryos) and *R. orientalis* (mice) (3). No cross-resistance with erythromycin or oleandomycin (3). Active in chick embryos and mice against psittacosis organism. Ineffective in mice infected with Rift Valley fever virus (4).

Toxicity: LD_{50} (mice) 200 to 250 mg per kg intravenously, >1000 mg per kg intraperitoneally (3).

References:

1. Shimo, M. *et al.* J. Antibiotics (Japan) **12A:** 1–6, 1959.
2. Tomosugi, T. *et al.* J. Antibiotics (Japan) **12A:** 7–11, 1959.
3. Komatsu, N. J. Antibiotics (Japan) **12A:** 12–16, 1959.
4. Komatsu, N. *et al.* J. Antibiotics (Japan) **12A:** 173–176, 1959.

Telomycin

Produced by: Streptomyces sp. (1).

Synonym: Antibiotic C 159.

Method of extraction: Broth-filtrate extracted with butanol at pH 8.4. Extract azeotropically distilled *in vacuo* to remove excess water and concentrate the antibiotic. Addition of Skellysolve B to concentrate gives precipitate. Filtered aqueous solution of solid at pH 7.5 to 8.0 reprecipitated by adjusting to pH 3.3. Solid extracted into water-saturated butanol. Extract concentrated *in vacuo*. Precipitate forms on addition of acetone to concentrate. Solid dissolved in 85 per cent aqueous methanol and reprecipitated with acetone (1).

Chemical and physical properties: Polypeptide. Pale cream or gray amorphous solid. Molecular weight, about 1000. Ultraviolet absorption spectrum maxima at 340 mμ ($E^{1\%}_{1cm}$ 127) and 260 to 280 mμ (broad low peak). C = 57.4%; H = 6.74%; N = 13.1%. No S. Most soluble in water above pH 8.5; least soluble at pH 3.3. Inorganic salts depress solubility in water. Moderately soluble in alcohols; very slightly soluble in acetone and ethyl acetate. Insoluble in ether, chloroform, and hydrocarbons. Heat- and pH-stable. Ninhydrin, biuret, and Fehling tests negative. Contains glycine, alanine, threonine, and aspartic acid (1).

Biological activity: Active on gram-positive bacteria (1.6 to 31.2 μg per ml). Not active on gram-negative bacteria or *C. albicans*. No cross-resistance with commonly used antibiotics. Active against *Staph. aureus* and *D. pneumoniae* infections in mice (2).

Toxicity: LD_{50} (mice) >1000 mg per kg orally, intravenously, intraperitoneally, or intramuscularly. Not absorbed orally (3).

References:

1. Misiek, M. *et al.* Antibiotics Ann. 1957–1958, pp. 852–855.
2. Gourevitch, A. *et al.* Antibiotics Ann. 1957–1958, pp. 856–862.
3. Tisch, D. E. *et al.* Antibiotics Ann. 1957–1958, pp. 863–868.

Tennecetin

Produced by: Streptomyces chattanoogensis.

Method of extraction: Extraction of broth-filtrate with n-butanol, concentration of solvent *in vacuo*, precipitation with anhydrous ether.

Chemical and physical properties: Tetraene. Soluble in water, methanol, 95 per cent ethanol, propylene glycol, pyridine, formamide, and n-butanol. Insoluble in chloroform, ethyl acetate, amyl acetate, ether, and petroleum ether. Aqueous solutions are alkaline. Solutions in water stable for at least 1 month in the refrigerator. Unaffected by light. Stable for 20 minutes at pH 7.0 at 100°C. Light-absorption maxima at 288, 300 to 302, and 315 to 318 mμ. Infrared absorption spectrum given in reference 1. If chromatographed on paper using wet butanol, tennecetin has different Rf values from rimocidin and nystatin. Wine-red color with concentrated sulfuric acid. Potassium permanganate is readily decolorized. No reaction with $FeCl_3$. Negative ninhydrin test (1).

Biological activity: Active against yeasts, filamentous fungi, and some bacteria, such as certain species of *Corynebacterium* and *Pseudomonas* (1).

Toxicity: LD_{50} (mice) 125 mg per kg intraperitoneally, 180 mg per kg subcutaneously. LD_{50} (rabbits) 20 mg per kg intravenously, 120 mg per kg intramuscularly, 68.5 mg per kg intraperitoneally, 180 mg per kg subcutaneously, and >200 mg per kg orally (2).

References:

1. Burns, J. and Holtman, D. F. Antibiotics & Chemotherapy **9:** 398–405, 1959.
2. Barr, F. S. Antibiotics & Chemotherapy **9:** 406–408, 1959.

Tertiomycin A

Produced by: Streptomyces eurocidicus (the same strain also produces eurocidin and azomycin); *S. albireticuli* (this culture also produces carbomycin, eurocidin, and enteromycin).

Method of extraction: I. Culture-filtrate adjusted to pH 2.0 and extracted with ethyl acetate; this extract contains azomycin. The pH of the culture is then adjusted to 7.0, and the culture extracted again with ethyl or butyl acetate; this second extract contains tertiomycin. Extract concentrated under reduced pressure to a syrup. Syrup extracted with pH 2.0 water. The aqueous solution extracted with ethyl acetate at pH 7.0. Ethyl acetate solution concentrated *in vacuo*. Residue extracted with ether; the ether solution concentrated *in vacuo*, leaving tertiomycin. Recrystallized from ethanol (1). II. Broth-filtrate extracted at pH 8.0 to 8.2 with butyl acetate. Extract washed with water and extracted with dilute HCl (pH 2.0). Extract adjusted to pH 6.5 and evaporated to dryness. Residue taken up in benzene; hexane added to incipient precipitation. Crystals of carbomycin are deposited on standing. Mother liquor evaporated *in vacuo*. Benzene solution of this residue extracted with acidic water (pH 1.0 to 2.0). Back-extracted into chloroform. Re-extracted into water. Aqueous extract adjusted to pH 9.0, and extracted with ether. Ether evaporated to dryness. Recrystallized from benzene-hexane (2).

Chemical and physical properties: White needle-shaped crystals. Browns at 208°C; melts at 215–

217°C (decomposition). Basic substance. Soluble in methanol, ethanol, butanol, ethyl acetate, butyl acetate, ether, dioxane, chloroform, and acetone. Insoluble in water and petroleum ether. C = 60.0%; H = 8.4%; N = 2.5%. No S or halogen detected. $C_{42}H_{69}NO_{16}$ (2). Negative ninhydrin, biuret, Benedict, Sakaguchi, xanthoproteic, $FeCl_3$, maltol, and Fehling tests. Positive Molisch and Seliwanoff reactions. Reddish orange color when added to 40 per cent sulfuric acid. Ultraviolet light-absorption at 233 mμ ($E^{1\%}_{1\,cm}$ 316) in ethanolic solutions. $[\alpha]^{17}_{D} = -44°$ (c = 1 per cent in ethanol) and −49° (c = 1 per cent in chloroform) (1). Paper chromatographic data given in reference 1. Black precipitate with Tollen's reagent. *Acetyl derivative:* m.p. 212–217°C (decomposition) or 210–215°C (decomposition). *Thiosemicarbazone:* m.p. 198°C (2).

Biological activity: Active against gram-positive bacteria. No activity against gram-negative bacteria, or fungi (1). *Acetyl derivative:* Less active than parent compound on *Staph. aureus* and *B. subtilis* (2).

Toxicity: Mice tolerate 415 mg per kg intraperitoneally (1).

References:

1. Osato, T. *et al.* J. Antibiotics (Japan) **8A:** 105–109, 1955.
2. Miyake, A. *et al.* J. Antibiotics (Japan) **12A:** 59–64, 1959.

Tertiomycin B

Produced by: Streptomyces eurocidicus.

Synonym: Related to antibiotic 446.

Method of extraction: Culture-filtrate extracted with butyl acetate at pH 7.5. Solvent evaporated *in vacuo* to syrup; washed with petroleum ether. Residue dried *in vacuo*; extracted with carbon tetrachloride, and the solvent solution passed through an alumina column. Elution with ethyl acetate. Evaporation of solvent. Residue dissolved in hot benzene; concentration and cooling of benzene solution yields crystalline tertiomycin B.

Chemical and physical properties: Basic substance. White needle-shaped crystals; m.p. 97–99°C. Light-absorption maxima at 231 ($E^{1\%}_{1\,cm}$ 237) and 278 mμ ($E^{1\%}_{1\,cm}$ 1.8) in ethanolic solution. $[\alpha]^{22}_{D} = -56°$ (c = 1 per cent in ethanol). Tentative empirical formula: $C_{43}H_{71}NO_{17}$. Negative Fehling, ninhydrin, Liebermann, and $FeCl_3$ reactions. Positive Tollen and Molisch reactions. Light reddish brown color with 40 per cent sulfuric acid.

Biological activity: Inhibits the growth of gram-positive bacteria at concentrations of 0.1 to 3 μg per ml. Very little activity against mycobacteria. Inactive against the gram-negative bacteria and fungi tested.

Toxicity: LD_{50} about 250 mg per kg intravenously (animal used not stated).

Reference: 1. Osato, T. *et al.* J. Antibiotics (Japan) **8A:** 161–163, 1955.

Tetracycline

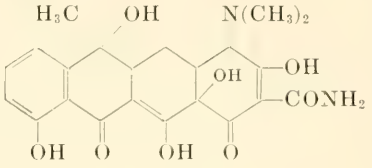
Produced by: Streptomyces aureofaciens strains (21, 25). (Tetracycline is produced instead of chlortetracycline when bromide is added to the medium to inhibit chloride utilization, or when the chloride ions are removed from the medium (19, 25).) *S. sayamaensis* (24) (under the same conditions as above), *S. viridifaciens* (21, 31), *S. feofaciens* (33), *Streptomyces* sp. (36, 38), *S. persimilis* (39), and catalytic dehalogenation of chlortetracycline (2, 4, 20).

Synonym: Antibiotic HA 20A (25).

Method of extraction: IA: Broth-filtrates, after treatment with sequestering agents to remove excess polyvalent metallic ions, are extracted with butanol or methyl isobutyl ketone at pH 8.5 in the presence of either 0.5 per cent $CaCl_2$ or a wetting agent such as the Tergitols or Aerosols. Organic extract concentrated, and mixed with Skellysolve C to precipitate the free base, or converted to an acid-salt. Purified by extracting with NH_4OH and chromatography on Florisil, washing with acetone or methanol, and elution with dilute acid (26, 31, 35). IB: Whole culture treated with oxalic acid, pH adjusted to 3.5 with NH_4OH, and filtered. N-(Lauroylcolaminoformylmethyl) pyridinium chloride ("Emulsept") in ethyl acetate is added to the filtrate, pH adjusted to 8.5, and the whole stirred. Organic layer extracted with acidic water (pH 1.7). Extract adjusted to pH 7.8 with NH_4OH to give tetracycline (25). IIA. Precipitated from broth at pH 10.0 with high molecular weight quaternary ammonium compounds such as the "Arquads" (see oxytetracycline). This precipitate, which is a complex salt composed of tetracycline, the organic base, and polyvalent metallic ions (either present in or added to broth), is slurried with water-chloroform at pH 10.5. Organic phase extracted with aqueous acid at pH 1.2 to 2.5 as in IB (25). IIB. The precipitated complex in IIA may be added to methanol, kept at pH 2.0 to 2.5, and $CaCl_2$ added. Addition of further acid gives the tetracycline acid-salt; raising the pH from 5 to 7 gives free tetracycline (25, 34). III. Whole culture acidified to pH 1.0 to 2.0 and filtered. Filtrate adjusted to pH 7.5 to 8.0 to precipitate crude tetracycline.

Aqueous suspension of precipitate acidified to pH 1 to 4, and NaCl and butanol added. Cooling gives hydrochloride crystals (33). IV. Precipitated from broth-filtrate at pH 8.5 to 9.5 on addition of $CaSO_4$, $BaCl_2$, or $MgCl_2$. Extracted into water at pH 1.5 and filtered. Filtrate adjusted to pH 8.0. This insoluble polyvalent metallic ion salt may be slurried with butanol or acetone at pH 2.0, filtered, and precipitated from the organic solvent by adding water and raising the pH to 4.2 (40). V. Precipitated from acidified broth-filtrate with such arylazosulfonic acid dyes as Polar yellow 5G, metanil yellow, Wool blue black 6B, or orange II (36). VI. Adsorbed from broth on IRC-50 (H^+ phase) and eluted with *N* HCl in 60 per cent methanol. Can also be adsorbed on Dowex 1, Dowex 50, or IR-112, and eluted with dilute alkali (25). VII. Purified by countercurrent distribution using water-saturated butanol at pH 3.0 and a butanol-saturated aqueous solution of the antibiotic (25). VIII. See I under chlortetracycline, "Method of Extraction."

Chemical and physical properties. Amphoteric substance. *Trihydrate of free base:* Yellow orthorhombic (25) or equant to tabular (32) crystals; m.p. 170–175°C (decomposition) (25). Swells and yellows at 165–170°C. Very soluble in methanol. Soluble in ethanol, butanol, ethyl acetate, and chloroform. Soluble to less than 1 mg per ml in water, benzene, and diethyl ether. Insoluble in petroleum ether. Least soluble in water at pH 6.0 (25, 32). *Anhydrous tetracycline:* m.p. 160–168°C (25). Ultraviolet absorption spectrum maxima: in 0.1 *N* HCl: 220 (E = 13,000), 268 (ϵ = 18,040), and 355 mμ (ϵ = 13,320); in 0.01 *M* methanolic HCl: 268 (log ϵ = 4.27) and 363 mμ (log ϵ = 4.14); in 0.01 *M* methanolic NaOH: 246 (log ϵ = 4.24) and 372 mμ (log ϵ = 4.20), or at 245, 265, and 382 mμ (3, 20); in methanol: 235 ($E^{1\%}_{1\,cm}$ 188), 269 ($E^{1\%}_{1\,cm}$ 271), 298.5 ($E^{1\%}_{1\,cm}$ 128), and 365 mμ ($E^{1\%}_{1\,cm}$ 272) (25). Infrared spectrum given in references 9, 25, and 33. $[\alpha]^{25}_{D}$ = −239° (c = 1 per cent in methanol) (2). Gives an orange-yellow solution in Ehrlich's *p*-dimethylamino-benzaldehyde reagent (oxytetracyline gives a blue-green precipitate and supernatant, and chlortetracycline, a canary-yellow solution). In H_2SO_4 it gives a stable violet color (oxytetracycline gives a cherry-red solution, chlortetracycline, a purple solution rapidly changing to greenish black) (9). Separation of the tetracyclines by paper chromatography has been reported (5). Rf values of tetracycline in various systems given in reference 26. Crystallographic data given in references 9, 25, and 32. Somewhat more stable than oxytetracycline and much more stable than chlortetracycline in aqueous solution (13). Most stable at acid pH (5, 8). $C_{22}H_{24}O_8N_2 \cdot 3H_2O$: C = 52.9%; H = 6.2%; N = 5.5%; H_2O = 10.9% (2). pK_a = 8.3 and 10.2 (2). Structural formula of tetracycline:



Hydrochloride: Bright yellow crystals. Darken gradually; melt with evolution of gas at 214°C (2, 20). Soluble in water to 100 to 200 mg per ml. Very soluble in methanol and ethanol; slightly soluble in 2-methoxymethanol and n-butanol; relatively insoluble in other organic solvents (32, 33). Infrared spectrum given in reference 25. $[\alpha]^{25}_{D}$ = −257.9° (c = 0.5 per cent in 0.1 *N* HCl) (2) or $[\alpha]^{29}_{D}$ = −283° (c = 0.65 per cent in water) (9). *Na and K salts:* Yellow crystals (33). *Ca salt:* Yellow crystals. Soluble in water to 890 μg per ml at pH 8.55 (32, 33). *Mg salt:* Soluble in water to 1 mg per ml at pH 7.0 (32).

Biological activity: Antibacterial activity is essentially the same as that of chlortetracycline and oxytetracycline (5, 8, 9). Not active on filamentous fungi or yeasts (8). Cross-resistance with the other tetracyclines (12). *In vivo:* Active in animals on *Streptococcus mitis*, *Staph. aureus*, *D. pneumoniae*, *Listerella monocytogenes*, *B. anthracis*, *Pasteurella multocida*, *K. pneumoniae*, and *Sal. typhosa* infections. Moderately active on *Streptococcus pyogenes*, *Sal. gallinarum*, and *M. tuberculosis* infections (6, 12). Under certain conditions, potentiates experimental infections with *C. albicans* and *Staph. aureus* in mice (14). Active on *Toxoplasma gondii* in mice (11), natural psittacosis infections in parakeets and pigeons (18), feline pneumonitis organism in chick embryos (23), and that of primary atypical pneumonia (Eaton) in cotton rats (15). Concentrations of 1:8000 inhibit the reproduction of the nematode *Anguillula aceti*. At 1:10,000 ascarid motility is stimulated, followed by a period of depressed activity. High concentrations are toxic (41). Some control of crown-gall (*Agrobacterium tumefaciens*) of roses (27). Active on bacterial wilt of sweet corn (*Bacterium stewartii*) (28). Inclusion in the diet improves feed efficiency, but not gain rates in pigs (10). Increases weight gain and feed efficiency in chicks (22). Tetracycline localizes in tumor and bone tissue and persists there, probably in the form of a loosely bound complex with a peptide (29).

Toxicity: LD_{50} (mice) 145 to 170 mg per kg intravenously, 330 to 355 mg per kg intraperitoneally, 400 mg per kg subcutaneously, 4250 mg per kg orally (7, 16, 25). LD_{50} (rats) 128 to 220 mg per kg intravenously, 320 mg per kg intraperitoneally, >3000 mg per kg orally (7, 16). LD_{50} (10-day-old chick embryos) 3.2 mg, via allantoic cavity (37). Minimal concentration causing mitosis inhibition in HeLa cells is 125 μg per ml (30). Highest concentration permitting epithelial cell migration in tissue culture is 140 μg per ml (42). Inhibits chlorophyll formation only at levels toxic to plant growth (17).

Utilization: Application clinically is essentially like that of chlortetracycline or oxytetracycline.

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Tetracyclines Derivatives

Produced by: Chemical transformation; *Streptomyces aureofaciens* (9).

Chemical and physical properties: I. Epimers of tetracycline and anhydrotetracycline are described in references 3, 4, and 7. Separation of the various tetracyclines and their epimers (quatrimycins) by paper chromatography (5, 6) and by countercurrent distribution (8) has been reported. II. Tetracycline-related compounds which have no biological activity are produced by *S. aureofaciens* (9). III. Derivatives, including anhydrochlortetracycline and 9-t-butylanhydrochlortetracycline among others, are described in reference 2.

Biological activity: Anhydrotetracyclines, as

well as the epimers of the tetracyclines and anhydrotetracyclines, are biologically active (1, 7). The derivatives referred to in III (above) are more active *in vitro* (0.78 to 6.25 μg per ml) against *Trichomonas vaginalis* than are the parent compounds. All are also active *in vivo* on *T. vaginalis*, especially 9-t-butylanhydrochlortetracycline. The derivatives are less effective than the parent compounds on *Endamoeba histolytica* infections. None has activity on *Trypanosoma equiperdum* in mice; 9-t-butylanhydrochlortetracycline is active, at high levels, against pinworm infections (*Syphacia obvelata*) in mice (2).

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Tetraene Antifungal Antibiotic

Produced by: Strains of *Streptomyces fungicidicus*.

Method of extraction: Extracted from the culture-filtrate with butanol.

Chemical and physical properties: Tetraene. Soluble in methanol, ethylene glycol, propylene glycol, benzaldehyde, glacial acetic acid, formaldehyde, pyridine, isopropyl alcohol. Slightly soluble in ethanol, butanol, propyl alcohol, and amyl alcohol. Insoluble in dioxane, toluene, xylol, ethyl and butyl acetate, anhydrous acetone, chloroform, ether, ligroin, hexane, and benzene. Ultraviolet absorption spectrum maxima at 290, 300, and 317 mμ. Positive Molisch, Fehling, and $KMnO_4$ reactions. Negative $FeCl_3$, Millon, Tollen, Schiff, and Sakaguchi reactions. Stable at acidic pH; unstable at alkaline pH.

Biological activity: Active on yeasts and filamentous fungi.

Reference: 1. Umezawa, H. *et al.* Japanese Patent 5744, 1956.

Tetrin

Produced by: Streptomyces sp.

Method of extraction: Can be adsorbed on carbon or cation exchange resins and eluted from the carbon with ethanol and methyl Cellosolve and from the resin with 10 per cent NaCl.

Chemical and physical properties: Tetraene. Very soluble in water and lower alcohols. Slightly soluble in chloroform. Insoluble in ether, acetone, and hydrocarbons. Ultraviolet absorption spectrum maxima at 291, 305, and 319 mμ (in water). Unstable at acidic pH; stable at neutrality and alkaline pH. Boiling for 1 hour reduces the activity 20 per cent; autoclaving at 121°C for 20 minutes destroys 55 per cent of the activity. Rf values on paper chromatography given in reference 1.

Biological activity: Active on filamentous fungi and yeasts. Inactive on bacteria. Active on leaf spot (*Cercospora sojina*) of soybean, but enhances rust (*Puccinia asparagi* D. C.) of asparagus.

Toxicity: Nontoxic when applied as a spray (1000 μg per ml) to tomato, soybean, beet, broccoli, radish, and pea plants. Similar treatment of lettuce, tomato, bean, pea, radish, and melon seeds is also nontoxic.

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Thermomycin

Produced by: Streptomyces thermophilus (S-form).

Method of extraction: Precipitated from broth by half-saturated $(NH_4)_2SO_4$ or extracted with ether.

Chemical and physical properties: Crude ether-extract: yellow powder. Heating for 15 minutes at 75°C reduces activity by 25 per cent; activity destroyed by heating for 15 minutes at 100°C. Nondialyzable. Passes Berkefeld-N and Seitz filters.

Biological activity: Active on *Corynebacterium diphtheriae*. Very weakly active on *Listerella monocytogenes*. Not active on other bacteria.

Reference: 1. Schöne, R. Antibiotics & Chemotherapy **1:** 176–180, 1951.

Thermoviridin

Produced by: Thermoactinomyces viridis.

Method of extraction: Filtered broth adjusted to pH 3 with phosphoric acid and held at 0–5°C for

2 hours. Precipitate thus formed is washed with water and extracted with 80 per cent acetone. Acetone-extract evaporated and water remaining is freeze dried. Crude antibiotic purified by fractional precipitation from a methanolic solution using ethyl ether, or by countercurrent distribution.

Chemical and physical properties: Probably an organic acid with no sulfur or phenyl groups. Countercurrent (butanol-pH 6.05 sodium phosphate buffer) and chromatographic studies (butanol-20 per cent sodium phosphate) indicate a single entity. Stable at pH 2 to 8 at 37°C for 21 hours. At pH 10, 50 per cent of activity lost after 8 hours. Biuret, ninhydrin, xanthoproteic, Molisch, and lead acetate (for sulfur) tests negative. Dialyzable. Precipitated with saturated ammonium sulfate. Ultraviolet absorption maximum at 268 to 272 mμ in water.

Biological activity: Active primarily on gram-positive bacteria, including *Clostridium*. Not active against mycobacteria, fungi, or *Nocardia*.

Toxicity: Intraperitoneal injection of 32 mg (25 units per mg) into 209 mice had no toxic effect after 3 weeks of observation (1 unit = minimal amount of thermoviridin per ml which inhibits growth of *Staph. aureus* 209P in broth for 24 hours at 37°C).

Reference: 1. Schuurmans, D. M. *et al.* Appl. Microbiol. **4**:61–66, 1956.

Thioaurin

Produced by: Streptomyces sp. (1, 2).

Synonym: Antibiotic HA 9 (2).

Method of extraction: Filtered broth extracted with ethyl acetate. I. Extract concentrated *in vacuo*, water added, and remaining ethyl acetate evaporated off. II. Tarry aqueous suspension decanted, after standing overnight. Residue extracted with carbon tetrachloride or chloroform. Extract back-extracted with water. Water extracted again with ethyl acetate and step II repeated. On cooling, crystals precipitate. Purification by countercurrent extraction (ethyl acetate-water) or by chromatography on silica gel from chloroform and development with ethyl acetate. Recrystallized from acetone or ethyl acetate (1, 2).

Chemical and physical properties: Neutral yellow crystals; m.p. 175–181°C (decomposition) (1, 2). At a pressure of 0.05 mm, sublimes very slowly at 110–140°C (m.p. of sublimate, 176–177.5°C) (2). $C_{14}H_{12}O_4N_4S_4$: C = 38.98%; H = 2.89%; N = 12.90%; S = 29.41% (1). Or $C_7H_6N_2O_2S_2$: C = 39.13%; H = 2.91%; N = 12.29%; S = 30.05% (2). Ultraviolet absorption spectrum maxima at 232 and 370 mμ (in 0.5 *M* HCl or methanol) and at 300 mμ (in 0.5 *M* NaOH) (1, 2). Heating in boiling 6 *N* HCl gives a dihydrochloride in the form of biologically active, yellow plates; m.p. 210–215°C (decomposition). Oxidation with 30 per cent H_2O_2 gives a yellow, biologically inactive, crystalline product; m.p. 205–210°C (1). Optically inactive Negative $FeCl_3$ test. Insoluble in water and chloroform; slightly soluble in ethyl acetate and benzene; fairly soluble in methanol. Rf on paper chromatography (wet n-butanol) = 0.7 to 0.79 (2). Crystallographic and infrared data given in reference 3.

Biological activity: Moderately active on gram-positive and gram-negative bacteria, and on some fungi and yeasts (1, 2). Activity not diminished by serum or cysteine.

Toxicity: LD_{50} (mice) 15 to 16 mg per kg intravenously, 20 mg per kg subcutaneously (1, 2).

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Thiolutin

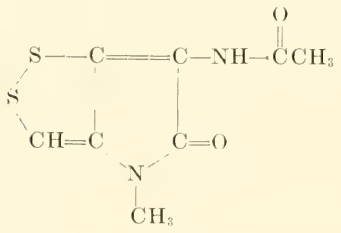
Produced by: Streptomyces albus (1, 12), *S. celluloflavus* (14), *Streptomyces* sp. (21) (the latter culture produces aureothricin simultaneously).

Synonym: Has the same 3-amino-5-methylpyrrolin-4-ono-(4,3-d)-1,2-dithiole nucleus as aureothricin (18).

Method of extraction: Whole culture adjusted to pH 2.0, heated at 90°C for 20 minutes, pH re-adjusted to 3.5, and the broth filtered using a filter-aid. Filtrate extracted with n-butanol, chloroform, methyl isobutyl ketone, ethyl acetate, benzene, or chloroform. Extraction can also be carried out at neutrality. Extract concentrated, then recrystallized from methanol, n-butanol, or dimethylformamide (12).

Chemical and physical properties: Neutral, optically inactive yellow crystals; m.p. 270°C (decomposition) (15). Sublimes without decomposition when heated at 200°C *in vacuo* (18). Soluble in butanol, dimethylformamide, chloroform, isopropanol, ethanol, methanol, acetone, glacial acetic acid, methyl isobutyl ketone, and pyridine. Limited solubility in petroleum ether, hexane, water, ether, and benzene (1, 12). λ_{max}^{EtOH} 388 mμ, with minor peaks at 311 and 250 mμ. Infrared spectrum given in reference 18. Optically inactive in glacial acetic acid (12). Stable in acidic and neutral solu-

tions; decomposes in alkaline solutions (1). Very thermostable (18). Desulfurization of thiolutin yields white crystalline desthiolutin, $C_6H_{14}N_2O_2$, which has no ultraviolet maximum above 220 mμ and is antibiotically inactive. Acid hydrolysis yields acetic acid and pyrrothine, $C_6H_6N_2OS_2$, a weak amine (also a hydrolysis product of aureothricin), which gives a characteristic red color with glutaconic aldehyde (2, 15, 18). Complete structure of thiolutin (3-acetamido derivative of 3-amino-5-methylpyrrolin-4-ono-(4,3-d)-1,2 dithiole):



Biological activity: Active against gram-positive and gram-negative bacteria in concentrations of 1 to 15 μg per ml. *Pseudomonas* and *Brucella* are resistant. Pathogenic fungi inhibited in concentrations of 1 to 5 μg per ml; *C. albicans* is resistant. Mild but definite microbicidal action against bacteria, fungi, and protozoa (1). Active on *Chlorella pyrenoidosa* at 5 μg per ml (22). Active *in vitro* on influenza A (PR 8) (23). Molluscacide (17). Active on fire blight (*Erwinia amylovora*) (3, 13), and moderately active on frog eye disease (*Physalospora abtusa*) of apple (13). Controls late blight of potato (*Phytophthora infestans*) (8), and has a protective effect against *Fusarium* wilt of tomato (4). Partial control of *Rhizoctonia* infection of lettuce and tomatoes, and *Verticillium* infection in tomatoes and potatoes (9). Control of tobacco blue mold (*Peronospora tabacina*) (16), and downy mildew of broccoli (*Peronospora parasitica*) (20). Moderate protection against *Helminthosporium* infection of oat seed; slight protection against cotton anthracnose (*Colletotrichum gossypii*) (5). Stimulates germinating seed growth of *Agave toumeyana* (6), oat, buffet grass, and Madrid sweet clover, and top growth of the latter three (11). Increases number of actinomycetes and bacteria in fallow soils to which it is added, and nodules on sweet clover grown in such soil, but decreases fungal population (11). Has a B_1-sparing effect on virus-tumor tissue of *Rumex acetosa* (7).

Toxicity: LD_{50} (mice) 25 mg per kg subcutaneously, 25 mg per kg orally (1). Not toxic at 200 ppm applied as spray to young tomato plants, or at 50 ppm as root solution to corn, pea, tomato, or sunflower plants, or at 120 ppm to carnation cuttings (19). Not inhibitory to germination of corn, pea, or radish seeds soaked in 100 ppm for 8 hours (5). Toxic at therapeutic levels to human spermatozoa (10).

Utilization: Plant disease. Plant growth stimulation.

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Thiomycin

Produced by: Streptomyces sp. closely related to *S. phaeochromogenes* var. *chloromyceticus*.

Method of extraction: Culture adjusted to pH 2.0 and filtered to remove the mycelium. Filtrate passed through a column of cation exchange resin, "Ionex C" (H^+ phase). Column washed with water and eluted with 80 per cent aqueous acetone. Eluate adjusted to pH 2.0 and concentrated *in vacuo*. The aqueous concentrate thus obtained is saturated with NaCl and extracted with ethyl acetate. Solvent extract concentrated to a syrup *in vacuo*. Syrup dissolved in chloroform and passed through a column of silica gel, which is then developed with chloroform. The antibiotic is collected in a deep yellow fraction. This fraction concentrated *in vacuo* to a syrup and then dissolved in ethyl acetate. Upon standing at room temperature, crude crystals are formed. Recrystallization from ethyl acetate solution.

Chemical and physical properties: Golden yellow needle-shaped crystals; m.p. 176–178°C (decomposition). Soluble in methanol, ethanol, butanol, acetone, ethyl acetate, chloroform, and benzene. Slightly soluble in water, ether, and petroleum ether. Negative ninhydrin, $FeCl_3$, and Fehling reactions. C = 49.6%; H = 5.5%; N = 8.9%; S = 16.3%. No halogen detected. Light-absorption maximum at 370 mμ ($E^{1\%}_{1cm}$ 375) in methanol solution and at 300 mμ ($E^{1\%}_{1cm}$ 555) in 0.5 *N* sodium hydroxide. Stable at pH 2.0 but unstable under alkaline conditions.

Biological activity: Inhibits the growth of gram-positive and gram-negative bacteria on nutrient agar at concentrations of 3 to 12 μg per ml at pH 6.0. Less active (25 to 51 μg per ml) at pH 6.8. Active against *M. smegmatis* 607. No activity against *Ps. aeruginosa*.

Toxicity: LD_{50} (mice) 10 mg per kg subcutaneously.

Reference: 1. Hinuma, Y. *et al.* J. Antibiotics (Japan) **8A:** 118–119, 1955.

Thiostrepton

Produced by: Streptomyces sp. This organism also produces two other antibiotics (1, 2).

Method of extraction: Mycelium extracted with chloroform, dioxane, or dimethylformamide. Extract concentrated *in vacuo*. Thiostrepton precipitates out of concentrate on addition of hexane. Purified by: (a) washing with hot benzene and crystallization from chloroform or dioxane on addition of 50 per cent aqueous methanol; (b) chromatography on alumina (chloroform as solvent and dioxane as developer); or (c) stirring a dioxane solution with carbon and precipitating the antibiotic with water (1, 2).

Chemical and physical properties: Weak base. White or very yellow crystals. Darkens at 235°C; m.p. 246–256°C (decomposition). Soluble in chloroform, dioxane, pyridine, benzyl alcohol, glacial acetic acid, and dimethylformamide. Hardly soluble in the lower alcohols. Insoluble in ether, acetone, hexane, benzene, ethyl and amyl acetate, dibutyl ether, water, dilute acids and bases. Soluble with loss of activity in dilute methanolic acids and bases. $[\alpha]_D^{23} = -98.5°$ (c = 1 per cent in glacial acetic acid); −61° (c = 1 per cent in dioxane); and −20° (c = 1 per cent in pyridine). Ultraviolet absorption spectrum shoulders at 225, 250, and 280 mμ (solvent not given) (1) or shoulders at 240, 280, and 305 mμ (methanol) (2). Infrared spectrum given in references 1 and 2. Positive biuret test. Negative ninhydrin and Sakaguchi tests. Forms complexes with alkaline earth metal salts and salts with acids. Both types of compounds are hydrolyzed to the free base when water is added. Most stable at pH 7.0. C = 51.3%; H = 5.4%; N = 14.6%; S = 7.4%; (1) or C = 51.75%; H = 5.3%; N = 15.84%; S = 9.22%; acetyl = 8.7% (2). No methoxyl groups. Equivalent weight, 380. Acid hydrolysis products include leucine (or isoleucine), valine, alanine, threonine, proline, lysine, glycine, aspartic acid, glutamic acid, and possibly cystine and tryptophan (1, 2).

Biological activity: Bacteriostatic. Active against gram-positive bacteria and mycobacteria (0.01 to 3.1 μg per ml). Relatively inactive (30 to 50 μg per ml) on gram-negative bacteria. No cross-resistance, using *Staph. aureus*, with commonly used antibiotics. Active on micrococcal and streptococcal infections in mice. Active against meningopneumonitis virus in eggs (1–3).

Toxicity: LD_{50} (mice) 41.7 mg per kg intravenously (2). Not absorbed from the gastrointestinal tract (1).

Utilization: Infections caused by gram-positive organisms. Intestinal sterilization (2).

References:

1. Pagano, J. F. *et al.* Antibiotics Ann. 1955–1956, pp. 554–565.
2. British Patent 795,570, May 28, 1958.
3. Kelley, J *et al.* Oral Surg., Oral Med., Oral Pathol. **12:** 1334–1339, 1959.

Totomycin

Produced by: Streptomyces crystallinus (1).

Method of extraction: I. Broth-filtrate extracted with n-butanol, amyl acetate, or amyl alcohol at pH 7.5. Adjusted to pH 2.2 to 2.5 with 10 per cent

aqueous H_2SO_4, and n-pentane added to separate an aqueous layer. Aqueous extract concentrated at room temperature under a fan. Addition of acetone precipitates impurities. II. Broth-filtrate extracted with n-butanol at pH 2.2. Extract filtered, neutralized, and pentane added. Aqueous layer treated as in I. III. Broth-filtrate stirred with Magnesol at harvest pH, filtered, then adsorbed on activated charcoal. Eluted with water-saturated n-butanol. Butanol concentrated *in vacuo*. Acetone added and mixture filtered. Solvent mixture from filtration concentrated *in vacuo* with the constant addition of water. Aqueous solution remaining after solvents have evaporated is lyophilized. Purified by partition chromatography on Dicalite with water as solvent and 85 per cent ethyl acetate-15 per cent n-butanol saturated with water as developer. Active fractions concentrated, decolorized with Darco, and filtered while hot. Addition of petroleum ether precipitates crude totomycin. Can also be chromatographed on Celite, with water as solvent and ethyl acetate-water-n-butanol (1:5:9) as developer.

Chemical and physical properties: Amorphous powder. Very soluble in water. Soluble in methanol and ethanol. Less soluble in β-methoxyethanol, 1,4-dioxane, n-butanol, formamide, hot pentanol, and dimethylformamide. Sparingly soluble in acetone, ethyl methyl ketone, and ethyl acetate. Insoluble in chloroform, ether, benzene, and petroleum ether. Infrared spectrum given in reference 1. C = 53.56%; H = 6.18%; N = 2.95%. $C_{21}H_{59}NO_{11}$. Distribution coefficients and Rf values in various systems given in reference 1. Most stable at acid or neutral pH. Completely destroyed at 100°C for 1 hour in 0.1 *N* HCl or 0.1 *N* NaOH.

Biological activity: Active on *Streptococcus hemolyticus* at 2 μg per ml. Much less active on other bacteria, including certain gram-negative bacteria (16 to 31 μg per ml). Not active on *B. cereus*, *B. subtilis*, *Sal. gallinarum*, *E. coli*, or *Ps. aeruginosa*. Active against *S. hemolyticus* infections in mice.

Toxicity: LD_{50} (mice) 1067 to 1154 mg per kg intraperitoneally, 1110 mg per kg intravenously.

Reference: 1. British Patent 758,276, October 3, 1956.

Toyocamycin

Produced by: Streptomyces sp. resembling *S. albus* (1), *S. toyocaensis* (3), *Streptomyces* sp. (4).

Synonyms: Antibiotic E 212 (2), antibiotic 1037 (4), unamycin B. Probably the same as monilin. Similar to vengicide.

Method of extraction: I. Broth adsorbed on Ionex C (carbonic acid ion exchange resin, H^+ form) at pH 7.6 to 8.0. Diluted with 80 per cent aqueous acetone (2, 3). Eluates are either: (a) Evaporated *in vacuo* at pH 5.0, then extracted with butanol at pH 8.0, and re-extracted into acidic water (pH 2.0). Water-extract evaporated *in vacuo* (pH 5.0), then extracted with ethyl acetate at pH 8.0. Extract concentrated *in vacuo* to dryness. Residue taken up in anhydrous methanol at 50°C. Cooling gives crystals. Recrystallized from anhydrous methanol. Or (b) evaporated to dryness *in vacuo*. Residue taken up in methanol. Ether added and the supernatant concentrated to dryness *in vacuo*. Crystallized as above. A small amount of toyocamycin can be extracted from the mycelium with 80 per cent aqueous acetone (2, 3). II. Extracted from broth-filtrate with butanol at pH 9.0. Concentration of extract gives crystals (4).

Chemical and physical properties: Weak base (2). *Anhydrous form* (from absolute methanol or acetone): colorless, fine needles; m.p. 243°C. *Hydrate:* Colorless prisms; m.p. 239–243°C (3). Soluble (3) or moderately soluble (2) in methanol, ethanol, n-butanol, ethyl acetate, dioxane, and acetone. Sparingly soluble in water and ether. Insoluble in benzene, chloroform, and petroleum ether (2, 3). Ultraviolet absorption spectrum maxima at 230 ($E^{1\%}_{1cm}$ 400) and 277 mμ ($E^{1\%}_{1cm}$ 548) in water; 235 ($E^{1\%}_{1cm}$ 760) and 273 mμ ($E^{1\%}_{1cm}$ 563) in 0.1 *N* HCl; 233 ($E^{1\%}_{1cm}$ 398) and 280 mμ ($E^{1\%}_{1cm}$ 571) in 0.1 *N* NaOH; and 230 and 279 mμ, with a weak maximum at 339 mμ (methanol) (2, 3). Infrared spectrum given in references 2 to 4. $[\alpha]_D^{35} = -51°$ (c = 0.13 per cent in water) (4). Conflicting reports on ninhydrin test (2, 3). Negative biuret, Sakaguchi, Fehling, $FeCl_3$, Molisch, Millon, Ehrlich, and Pauly tests. Relatively stable at pH 5.0, but unstable at pH 2.0, 7.0, or 9.0 (2). C = 49.63%; H = 4.79%; N = 24.14%. $C_{12}H_{14}N_5O_4$ (3). Toyocamycin has the same structure as tubercidin plus a nitrile group (6).

Biological activity: Inhibits *Candida* at 1.0 to 12.5 μg per ml. Slightly active (6.3 μg per ml) on *Zygosaccharomyces* and *Aspergillus oryzae* (12.5 μg per ml). Also active on *M. tuberculosis* var. *hominis* H37Rv at 2 μg per ml, but not on other mycobacteria, bacteria, or fungi. Conflicting reports on anti-*Trichomonas vaginalis* activity (2, 4). Slight inhibitory effect on Ehrlich carcinoma (ascitic) (2, 3). Active on influenza A (PR 8) *in vitro*, probably by direct inactivation of the virus particle (5).

Toxicity: Mice tolerate 5 mg per kg intrave-

nously; 10 mg per kg produces delayed death with abnormality of the liver. Toxicity decreased by simultaneous administration of DL-methionine or glucuronic acid (2). LD_{50} (mice) 10 to 20 mg per kg subcutaneously (3).

References:

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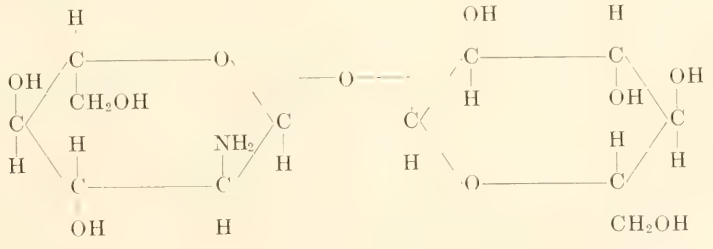
Trehalosamine

Produced by: Streptomyces sp. resembling *S. lavendulae* (1).

Method of extraction: Active fraction adsorbed from broth-filtrate by activated carbon; eluted with water at pH 2.5. Readsorbed on IR-120 and eluted with ammonium hydroxide. Eluate concentrated *in vacuo*, adjusted to pH 5, diluted with methanol, and precipitated with acetone. Purification by chromatography on Al_2O_3 and elution with 70 per cent aqueous methanol. Recrystallized from anhydrous methanol (1, 2).

Chemical and physical properties: Amino disaccharide. *HCl:* White crystalline powder. $[\alpha]_D$ = +176° (c = 2.4 per cent in water). *Basic group:* pK_b = 6.9. $C_{12}H_{23}O_{10}N \cdot HCl$. Positive ninhydrin test. Negative Fehling, Benedict, and ammoniacal silver nitrate tests. Infrared spectrum given in reference 2. No ultraviolet absorption. Stable to dilute acid and alkali under mild conditions. Ammonia is liberated by boiling with strong alkali. *Octaacetyl monohydrate derivative:* m.p. 99–102°C. Ultraviolet absorption spectrum maximum of the acid degration product at 284 mμ. Trehalosamine is α-D-glucopyranosido-2-desoxy-2-amino-α-D-glucopyranoside (1, 2):



Biological activity: Very slightly active on gram-positive bacteria, including mycobacteria, and certain gram-negative bacteria (*E. coli* but not *Klebsiella friedländeri*) and yeasts. Not active on *Endamoeba histolytica* (1). Antimycobacterial activity antagonized by valicin, esculin, and trehalose. Action of trehalose is competitive in certain cases (3).

Toxicity: LD_{50} (mice) 5.4 gm per kg (1).

References:

1. Arcamone, F. *et al.* Giorn. microbiol. **2:** 205–214, 1956.
2. Arcamone, F. and Bizioli, F. Gazz. chim. ital. **87:** 896–902, 1957.
3. Ghione, M. and San Filippo, A. Giorn. microbiol. **3:** 189–196, 1957.

Trichomycin

Produced by: Streptomyces hachijoensis strains, resembling *S. rubrireticuli* (1, 5, 7); *Streptomyces* sp. (10).

Synonym: Belongs to the same group as candicidin and ascosin.

Method of extraction: I. Extracted from mycelium with warm acetone, methanol, or ethanol. Best method: 80 per cent acetone at pH 7.4 to 8.0. Extract concentrated *in vacuo*, and aqueous residue adjusted to pH 5.4 to precipitate yellow trichomycin. Purified by countercurrent distribution (chloroform-methanol-pH 8.5 Sörensen's buffer, 1:1:0.5). Active fractions concentrated *in vacuo* and adjusted to pH 5.4. II. Extracted from culture-filtrate by adsorption on 1 per cent Japanese acidic clay and elution with butanol-methanol-water (4:1:1). Eluates concentrated *in vacuo* and dried (2). III. Acetone-extracts of mycelium purified by chromatography on alumina (developer: pyridine-butanol-water, 3:4:7); 20 per cent ammonium sulfate added to active fractions. Benzene added to upper layer. Lower layer freeze dried to yield trichomycin (14). Precipitated from warm pyridine-dioxane-water-butyl acetate (30:40:52:18) on chilling. Precipitated from cold alkaline water with acetone (17). Chromatographed on alumina from water (pH 8.5 to 9.0) to give three fractions, A, B, and C. Development

with 0.067 *M* (pH 8.0) Sörensen's phosphate buffer gives Fraction A. B can be eluted with 20 per cent pyridine-5 per cent HCl (95:5), and C with pyridine-butanol-water (3:4:7). Fraction A precipitates as the 2,5-diamino-7-ethoxyacridine (rivanol) salt. B is purified by adding 20 per cent ammonium sulfate solution-butanol (25:5) to active fraction to force it into the butanol layer. B precipitates on addition of benzene to butanol. Fraction C concentrated, and 20 per cent ammonium sulfate added. Lower layer lyophilized to give C (15). Fractions further purified by countercurrent distribution (chloroform-methanol-pH 8.4 borate buffer, 2:2:1) to give pure A and B (24).

Chemical and physical properties: Complex composed of three biologically active components, A, B, and C. Acidic conjugated heptaenes, possibly containing a lactone group. *Complex:* Yellow substance. Decomposes at about 155°C, turning brick-red. Cannot be sublimed. Very soluble in propylene glycol, benzyl alcohol, pyridine, acetic acid, phenol, and the following when water is present: butanol, ethylene glycol monomethyl ether, dioxane, propanol, and methyl ethyl ketone. Moderately soluble when water is present in acetone, methanol, and ethanol. Very slightly soluble in water. Insoluble in xylene, ether, ethyl acetate, benzene, toluene, carbon disulfide, petroleum ether, ligroin, chloroform, and CCl_4 . Most soluble at alkaline pH. Ultraviolet absorption spectrum maxima at 286, 346, 364, 384, and 405 mμ (ethanol). These peaks are flattened to a plateau at 320 to 350 mμ or to two peaks at 235 and 335 mμ in water. Infrared data given in reference 5. Positive diazo, carbylamine, and Ehrlich tests. Negative ninhydrin, biuret, Fehling, Benedict, Molisch, Tollen, and quinone tests. Blue color in concentrated HCl or H_2SO_4 turns purple on standing. Most stable at pH 6 to 7. Heat- and photo-labile. Destroyed by ultraviolet light. Precipitated by bivalent metals, procaine, dihydrostreptomycin, and acrinol. No S or halogens. Conflicting reports on presence of N (see under components below). Ozonolysis products include acetone, glyoxal, and *p*-acetaminobenzoic acid. Oxidation with $KMnO_4$ yields *p*-aminoacetophenone and oxalic acid (1, 2, 5–7, 16–18, 22–24). *Components A and B:* Light yellow amorphous powders which turn deep yellow at 150–160°C and brown-yellow at 180°C. Do not melt or decompose when heated to 320°C. Soluble in glacial acetic acid, pyridine, dimethylformamide, and alkaline water. Insoluble in petroleum ether, ether, benzene, chloroform, and carbon disulfide. Positive Ehrlich (yellow), Ehrlich-Harter (red), diazo (red), $FeCl_3$ (pale violet), Carr-Price (blue-violet), Bayer, bromine, carbylamine, and dithiocarbamate tests. Uncertain reactions with 2,4-dinitrophenylhydrazine, Tollen, and Molisch tests. Contain C, H, O, and N. Negative ninhydrin, Fehling, and Benedict tests. Ultraviolet absorption spectrum maxima at 235, 340, 358, 377, and 400 mμ. Infrared spectrum given in reference 24. Fraction B in dilute aqueous alkali forms a precipitate at pH 9.0; A at pH 10.0 (24).

Biological activity: Active on *Trichomonas*, *Endamoeba histolytica*, *Treponema pallidum*, and *Trypanosoma cruzi*. Active on yeasts, fungi, and anaerobic bacteria, but not on aerobic bacteria. Activity reduced by serum. Resistance to trichomycin accompanied (in *Candida*) by decreased virulence for mice (1–3, 5, 9, 13, 16). *In vivo:* Active (mice) on *Trichomonas vaginalis*, *C. albicans*, *Treponema pallidum* (rabbits), *Endamoeba histolytica* (mice), and *Trichophyton asteroides* (guinea pigs) (3, 5, 8, 20, 21). Prolongs survival time in mice infected with *Cryptococcus neoformans* (25).

Toxicity: LD_{50} (mice) 2.2 mg per kg intraperitoneally and intravenously, and 17 mg per kg subcutaneously (12). Mice tolerate 300 mg per kg orally, but not 1 gm per kg (4).

Utilization: Vaginal candidiasis. *Trichomonas vaginalis* infections (3, 5). Otitis media caused by *Candida* spp. or *Absidia ramosa* (11). Trichophytosis (8).

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15. Hosoya, S. *et al.* J. Antibiotics (Japan) **8A:** 48–50, 1955.
16. Ito, T. *et al.* J. Antibiotics (Japan) **8A:** 103, 1955.
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18. Hattori, K. *et al.* J. Antibiotics (Japan) **8B:** 312–315, 1955.
19. Hosoya, S. and Hamamura, N. J. Antibiotics (Japan) **8B:** 417–418, 1955.
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22. Hosoya, S. and Hamamura, N. J. Antibiotics (Japan) **9A:** 129–131, 1956.
23. Nakano, H. *et al.* J. Antibiotics (Japan) **9A:** 172–175, 1956.
24. Hattori, K. *et al.* J. Antibiotics (Japan) **9A:** 176–181, 1956.
25. Fujino, T. *et al.* Med. J. Osaka Univ. **8:** 579–584, 1958.

Tubercidin

Produced by: Streptomyces sp.

Method of extraction: Broth-filtrate treated with charcoal at pH 8.0. Elution with 80 per cent acidic aqueous acetone. Acetone evaporated *in vacuo*. Aqueous residue washed with butanol, adjusted to pH 8, and extracted with butanol. Extract concentrated *in vacuo*, then cooled to precipitate tubercidin. Crystallized from boiling water.

Chemical and physical properties: Basic substance; m.p. 247–248°C (decomposition). Insoluble in acetone, ethyl acetate, chloroform, benzene, and petroleum ether. Sparingly soluble in water, methanol, and ethanol. Very soluble in water above pH 10 or below pH 4. Stable at pH 2 to 10 when boiled for 5 hours. $C_{11}H_{14}N_4O_4$: C = 49.76%; H = 5.31%; N = 21.21%. *Picrate:* m.p. 229–231°C (decomposition). pK_a = 5.2 to 5.3 (10°C). Ultraviolet absorption spectrum maxima at 227 and 270 mμ in acidic solution, and at 270 mμ in neutral or alkaline solution. Infrared spectrum given in reference 1. Positive diazo, Nessler, benzol, Bial (pentose), and Wheeler-Tollen (pentose) tests. Negative $FeCl_3$, Liebermann, Denige (tertiary alcohol), Fehling, Tollen, and sodium nitroprusside tests. Negative Molisch test becomes positive on acid hydrolysis. 4-Amino-7-(D-ribofuranosyl)-pyrrolo-(2,3-d)-pyrimidine (2, 3).

Biological activity: Active on mycobacteria, but not on other bacteria. Very slight activity on *C. albicans*. Active *in vitro* on NF mouse sarcoma, but not on Ehrlich ascites carcinoma *in vivo*.

Toxicity: LD_{50} (mice) about 45 mg per kg intravenously.

References:

1. Anzai, K. *et al.* J. Antibiotics (Japan) **10A:** 201–204, 1957.
2. Suzuki, S. and Marumo, S. J. Antibiotics (Japan) **13A:** 360, 1960.
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Tubermycins

Produced by: Streptomyces misakiensis.

Method of extraction: Broth-filtrate extracted with ethyl acetate at pH 2.4. Re-extracted into 0.2 *N* NaOH. Aqueous extract adjusted to pH 2.4 and extracted with ether. Extract concentrated to dryness *in vacuo*. Crude residue taken up in acetone and chromatographed on alumina (80 per cent aqueous acetone as developer) to separate tubermycins A and B. Crystallized from aqueous acetone.

Chemical and physical properties: Tubermycin A: Monobasic acid. Long, fine, yellow needles; m.p. 174°C. Ultraviolet absorption spectrum maxima (methanol) at 256 ($E^{1\%}_{1cm}$ 3510) and 365 mμ ($E^{1\%}_{1cm}$ 570), which shift to 254 and 370 mμ in acidic methanol. Infrared spectrum given in reference 1. Equivalent weight, 282. pK_a = 7.6. One C—CH_3 group. $C_{17}H_{16}N_2O_2$: C = 72.9%; H = 5.38%; N = 9.9%. Probably an alkyl-substituted phenazine with a carboxyl function. *Tubermycin B:* Phenazine-α-carboxylic acid. Yellow needles; m.p. 243°C. Ultraviolet absorption spectrum maxima (methanol) at 251 ($E^{1\%}_{1cm}$ 3790) and 364 mμ ($E^{1\%}_{1cm}$ 600), shifting to 249 mμ and 367 mμ in acidic methanol, but unchanged in alkaline methanol. Infrared spectrum given in reference 1. $C_{13}H_8N_2O_2$: C = 69.51%; H = 3.5%; N = 12.29%; no C—CH_3 group. Equivalent weight, 230. pK_a = 7.0. Both A and B are very soluble in alkaline water and sulfuric acid (red solution). Moderately soluble in acetone, acetic acid, dioxane, and dimethylformamide. Sparingly soluble in methanol, ethanol, ethyl acetate, benzene, and ether. Hardly soluble in water. Positive hydroxamic acid test. Negative Fehling, $FeCl_3$, maltol, ninhydrin, and diazo reactions.

Biological activity: Tubermycin A: Active on mycobacteria at 1.25 to 20 μg per ml. *Tubermycin B:* Active on mycobacteria at 10 to 50 μg per ml. Inactive against bacteria and fungi. Serum depresses activity somewhat.

Toxicity: Na salts: LD_{50} (mice) 160 mg per kg (A) and 400 mg per kg (B) intravenously.

Reference: 1. Isono, K. *et al.* J. Antibiotics (Japan) **11A:** 264–267, 1958.

Unamycin

Produced by: Streptomyces fungicidicus.

Method of extraction: Adsorption on charcoal. Charcoal washed with water and eluted with 70 per cent acetone at pH 9.0. Eluate concentrated *in vacuo*. Concentrated solution extracted with ethyl acetate. Extract adjusted to pH 3.0 and stored overnight in ice-box; precipitate forms. Precipitate dissolved in water at pH 8.4. On addition of acetone to 85 per cent, a precipitate occurs and is removed. The acetone is concentrated *in vacuo*. Concentrate adjusted to pH 3.0 and stored in the cold; crude crystals form. These are washed with ethanol, dissolved in 80 per cent aqueous methanol, concentrated *in vacuo*, and cooled; unamycin crystals form. Cation exchange resins can also be used to purify this antibiotic (1).

Chemical and physical properties: Acidic tetraene. White needle-shaped crystals. Becomes reddish above 140°C and decomposes at 148–150°C. Soluble in formamide, dimethylformamide, methanol, aqueous ethanol, aqueous propanol, aqueous butanol, aqueous acetone, aqueous dioxane, pyridine, glacial acetic acid, and alkaline water. Slightly soluble in isopropanol and butanol. Insoluble in acetone, dioxane, propylene glycol, ether, benzene, petroleum ether, and acidic water. Light-absorption maxima at 290 ($E^{1\%}_{1cm}$ 643), 304 ($E^{1\%}_{1cm}$ 1010), and 319 mμ ($E^{1\%}_{1cm}$ 875), with a shoulder at 279 mμ ($E^{1\%}_{1cm}$ 328). Infrared spectrum, which is given in reference 1, contains a band at 6.4 mμ. C = 52.24%; H = 7.77%; N = 1.74%. No halogen or S. $[\alpha]_D^{25} = -92°$ (c = 1 per cent in 80 per cent aqueous methanol). $[\alpha]_D^{28} = +79.2°$ (c = 1 per cent in dimethylformamide). Negative $FeCl_3$, Millon, Fehling, and Tollen tests. Positive Molisch test. Permanganate and bromine solutions are decolorized. Schiff test gives a light pink color, and sulfuric acid gives a reddish brown color turning black. Rf = 0.94 with 50 per cent aqueous acetone, and Rf = 0.68 with water-saturated butanol (1).

Biological activity: Active against many fungi but not bacteria. Examples of minimal inhibitory concentrations, in μg per ml: *C. albicans*, 1.56; *Aspergillus niger*, 1.56; *Microsporum audouini*, 3.13; *Sacch. cerevisiae*, 6.25. Fungicidal effect observed against *C. albicans* and *Cryptococcus neoformans*. Weak inhibition of *Trichomonas vaginalis*. Some effect *in vivo* against *C. albicans* infection in mice (2).

Toxicity: LD_{50} (mice) 89.6 mg per kg intravenously (2).

References:

1. Matsuoka, M. and Umezawa, H. J. Antibiotics (Japan) **13A:** 114–120, 1960.
2. Matsuoka, M. J. Antibiotics (Japan) **13A:** 121–124, 1960.

Unamycin B

Produced by: Streptomyces fungicidicus.

Synonyms: Related to toyocamycin, antibiotic E 212, and vengicide.

Method of extraction: Culture-filtrate adjusted to pH 8.0 and passed through a column of Duolite-S30 (H^+ form). Elution with 80 per cent aqueous acetone containing 2 per cent HCl. Eluate concentrated *in vacuo*. Concentrate adjusted to pH 9.0 and extracted with butanol. Back-extraction in water at pH 2.0. Methanol added to the aqueous extract and the mixture cooled, resulting in the formation of crude crystals. Recrystallization from water.

Chemical and physical properties: Basic substance. White needle-shaped crystals; m.p. 236–238°C (decomposition). Soluble in acidic water, acidic alcohol, and formamide. Slightly soluble in methanol, ethanol, butanol, and ethyl acetate. Insoluble in dioxane, toluene, acetone, ligroin, hexane, benzene, carbon tetrachloride, and glacial acetic acid. Light-absorption maxima in 0.05 *N* HCl at 236 ($E^{1\%}_{1cm}$ 626.5) and at 273 mμ ($E^{1\%}_{1cm}$ 458.8). Infrared absorption spectrum given in reference 1. C = 46.6%; H = 4.35%; N = 22.52%. $[\alpha]_D^{15} = -43°$ (c = 1 per cent in acidic methanol). Rf, using 90 per cent aqueous acetone = 0.89. Negative $FeCl_3$, Millon, Fehling, Tollen, Schiff, and Sakaguchi tests. Decolorization of permanganate and bromine solutions. Green Molisch reaction (1).

Biological activity: Narrow antifungal spectrum. Examples of minimal inhibitory concentrations, in μg per ml. *C. albicans*, 3.13; *Cryptococcus neoformans*, >100; *Aspergillus niger*, >100; *P. chrysogenum*, 3.13. Very little activity against most bacteria. Active against *M. phlei* (3.13 μg per ml) (2).

Toxicity: LD_{50} (mice) 10 mg per kg intravenously. Delayed toxicity observed.

References:

1. Matsuoka, M. and Umezawa, H. J. Antibiotics (Japan) **13A:** 114–120, 1960.
2. Matsuoka, M. J. Antibiotics (Japan) **13A:** 121–124, 1960.

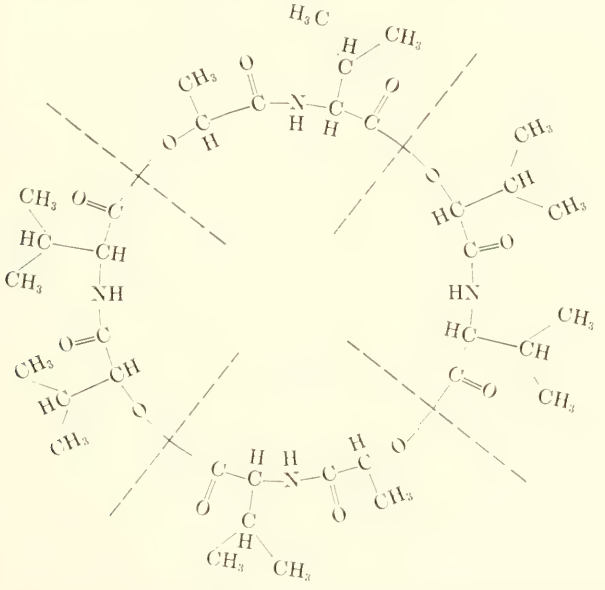
Valinomycin

Produced by: Streptomyces fulvissimus (1).

Synonyms: Aminomycin (3, 5). Similar to amidomycin.

Method of extraction: Powdered mycelium extracted in a Soxhlet apparatus with petroleum ether for 5 days, ether, acetone (2 days each), and 80 per cent methanol (5 days), in succession. Petroleum ether extract evaporated. Valinomycin precipitates slowly from the residual oil. Precipitate is fractionally crystallized from n-dibutyl ether. Some additional valinomycin is obtained from the methanolic extracts (1).

Chemical and physical properties: Polypeptide. Colorless rectangular plates; m.p. 190°C. Does not sublime at low absolute pressure. Readily soluble in common organic solvents; practically insoluble in water, 2 *N* HCl, and 2 *N* aqueous alkali. $\lambda_{max}^{n=hexane}$ 281 mμ. Infrared spectrum given in reference 3. $[\alpha]_D^{20}$ = +31.0° (c = 1.6 per cent in benzene). No color in concentrated H_2SO_4. Negative ninhydrin test. C = 58.34%; H = 8.25%; N = 7.48%; $C_{36}H_{60}O_{12}N_4$. Hydrolysis products include D- and L-valine, lactic acid, and α-hydroxyisovaleric acid. Structural formula (2, 4):



Biological activity: Active on the following organisms (minimal inhibitory concentration): *Staph. aureus* (1:30,000), *B. subtilis* (1:20,000), *M. tuberculosis* (human, virulent) (1:900,000). Active *in vivo* on *Trypanosoma brucei* (mice) at half the maximal tolerated dose (2, 3).

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1. Lindenbein, W. Arch. Mikrobiol. **17:** 361–383, 1952.
2. Brockmann, H. and Schmidt-Kastner, G. Chem. Ber. **88:** 57–61, 1955.
3. Brockmann, H. and Schmidt-Kastner, G. German Patent 926,806, April, 1955.
4. Brockmann, H. and Geeren, H. Ann. Chem. Liebigs **603:** 216–231, 1957.
5. Schmidt-Kastner, G. Personal communication. January 19, 1959.

Vancomycin

Produced by: Streptomyces orientalis (3). Certain strains produce one or more antibacterial substances other than vancomycin (9).

Synonym: Similar to ristocetin.

Method of extraction: I. Adsorbed at pH 7 to 8 from broth-filtrate on Permutit DR (OH^- phase). Eluted with 1 per cent glacial acetic acid in 30 per cent ethanol or acetone. Eluate concentrated *in vacuo*, and aqueous residue adsorbed on carbon at pH 5 to 7. Eluted with 50 per cent aqueous ethanol at pH 2.0 (sulfuric acid). Eluate concentrated *in vacuo*, with $Ba(OH)_2$ added to maintain pH at >2.5. Precipitated as the picrate, then converted to the hydrochloride; precipitated from ethanol by ether or acetone, or by concentration to dryness *in vacuo*. Purified by (a) passing through a mixed IR-120 (H^+ form)-IR45 (OH^- form) column. Effluent concentrated to give free base. Or (b) neutralizing a 20 per cent methanolic solution of the hydrochloride with 10 per cent NaOH to pH 8.0 at 25°C to give the free base (1, 9). II. Co-precipitated or adsorbed from broth on divalent metal hydroxides generated *in situ* as follows: 1 per cent $ZnSO_4$ added to broth filtrate, and pH adjusted

to 9.2 to give precipitation of vancomycin on or with the $Zn(OH)_2$ formed. Vancomycin removed by conversion to the water-soluble oxalate, the zinc oxalate being insoluble. Treated as in I (9). III. Adsorbed on low cross-linked Dowex 50 (H^+ and Na^+ forms) from broth-filtrates. Eluted with 30 per cent acetone containing 1 per cent triethylamine. Eluates concentrated and acidified to pH 3.2. Hydrochloride treated as in I (9).

Chemical and physical properties: Complex containing two components. *Free base:* Amphoteric substance containing organic chlorine. Colorless rosettes. Very soluble in water, dimethyl sulfoxide, dimethylacetamide, and other polar, water-miscible solvents. Moderately soluble in aqueous methanol. Insoluble in organic solvents, other than those above. Forms salts with orange II, methyl orange, and *p*-(*p'*-hydroxyphenyl)azobenzene-sulfonic acid. Precipitated from an aqueous solution with phosphomolybdic or phosphotungstic acids and heavy metals. Ammonium sulfate and sodium chloride precipitate it from acidic solutions. Forms solvates. Titration data given in reference 9. C = 54.91%; H = 5.85%; N = 8.66%; Cl = 4.27%; carbohydrate = 16 to 17%. No S or P (1, 8, 9). *Sulfate:* Crystalline state lost on drying. Very soluble in 50 per cent methanol containing 10 per cent urea. Insoluble in methanol. Ultraviolet absorption spectrum maxima at 278 ($E^{1\%}_{1cm}$ 38.7) (in acid) or 300 mμ ($E^{1\%}_{1cm}$ 41.3) (in alkali). *Hydrochloride:* White amorphous substance. Soluble in water; moderately soluble in aqueous methanol and phenol; insoluble in other organic solvents. Ultraviolet absorption spectrum maximum at 280 to 282 mμ (in acid), shifting to 303 to 305 mμ (in alkali). Infrared spectrum given in reference 1 and data in reference 8. Positive biuret, xanthoproteic, Molisch, Seliwanoff, anthrone (carbohydrate), and Folin-Ciocalteau (phenol) tests. Negative ninhydrin, Barfoed, glucose oxidase coupled dye oxidation (for glucose), Sakaguchi, Elson-Morgan, Fehling, Benedict, orcinol, HCl, Tollen phloroglucinol, Tollen naphthoresorcinol, Liebermann, and Ehrlich (aldehyde) tests. Green-brown color with $FeCl_3$ test. Paper chromatographic behavior given in reference 8. Isoelectric point about pH 5.0. Most stable at pH 3 to 5 and at low temperatures. Not destroyed by a variety of enzymes (1, 8, 9). Contains carboxyl, amino, and phenolic groups. Molecular weight, 3300. Hydrolysis with acid or alkali gives two ninhydrin-positive substances: one, aspartic acid, the other unknown, which gives an immediate atypical brown color with ninhydrin, slowly changing to blue. Partial hydrolysis with dilute acid gives glucose (9).

Biological activity: Active on gram-positive bacteria, mycobacteria, and some spirochetes. Slightly active on certain gram-negative bacteria. Not active on fungi (1). Bactericidal, acting only on multiplying bacteria (2). Less active on a weight basis than erythromycin and penicillin (8). Activity unaffected by pH. Resistance develops slowly. No cross-resistance with erythromycin, spiramycin, antibiotic E 129, or novobiocin (4). Active *in vivo* against *Staph. aureus*, *Streptococcus pyogenes*, *D. pneumoniae* type I, and *Borrelia novyi* infections in mice treated parenterally (1). No effect (mice) on *Toxoplasma gondii*, herpes simplex, or vaccinia viruses (5). Biological activity of the second, minor component of the complex is not known (9).

Toxicity: LD_{50} (mice) 489 to 700 mg per kg intravenously, 1734 mg per kg intraperitoneally, 5 gm per kg subcutaneously, with sloughing and necrosis, and 5 gm per kg orally. LD_{50} (rats) 319 mg per kg intravenously, 221 mg per kg intraperitoneally. LD_{50} (guinea pigs) 737 mg per kg intraperitoneally. Repeated intravenous doses cause thrombosis and edema in monkeys (6, 8). Highest concentration permitting epithelial cell migration in tissue culture is 8.1 mg per ml (11).

Utilization: Antibiotic-resistant staphylococcal or streptococcal infections (10). Clinical trials indicate that use of the drug would be limited by side effects such as irritation of tissues, drug-induced fever, and possible renal irritation (7).

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Vengicide

Produced by: Streptomyces vendargensis which also produces five or six other antibiotics, including oxytetracycline.

Synonyms: Similar to toyocamycin, monilin, and unamycin B.

Method of extraction: I. Whole culture acidified to pH 2.2 and filtered. Adsorbed from filtrate by activated carbon at pH 6.5 to 7.5. Eluted with water-saturated butanol containing 1 per cent phenol at pH 1.5. Eluate concentrated *in vacuo* at pH 2.5. Residue extracted with butyl or amyl alcohol at pH 8 to 11. Back-extracted into water at pH 1.5 to 2.5. Oxytetracycline present precipitated with a quaternary ammonium base. Filtrate extracted with butanol at pH 9 to 10, then re-extracted into water at pH 2. Adjustment of aqueous extract to pH 4.0 to 5.0 gives vengicide. Decolorized with carbon and reprecipitated from water. II. Broth-filtrate obtained as above concentrated by evaporation, and extracted with butanol or amyl alcohol at pH 8 to 11. III. Culture-filtrate obtained as in I, and treated with the ion exchange resin Duolite S30 at pH 2.0 to 2.5 to remove the oxytetracycline. Vengicide then purified as in I or II.

Chemical and physical properties: Amphoteric substance. White monoclinic crystals; m.p. 241.5–243°C. Slightly soluble in water (0.4 mg per ml at room temperature), acetone, methanol, butanol, and amyl alcohol. Insoluble in diethyl ether. Ultraviolet absorption spectrum maxima at 233.5 and 273.5 mμ (in 0.05 *N* HCl). Infrared spectrum given in reference 1. $[\alpha]_D^{20} = -51.6°$ (in 0.1 *N* HCl); pH of the saturated solution is about 6.3. C = 47.05%; H = 4.85%; N = 23.25%. $C_{24}H_{29}O_9N_{10}$. Crystallographic data given in reference 1.

Biological activity: Active at 5 mg per ml on *Blastomyces sulfureum, B. dermititidis, Histoplasma capsulatum*, and *C. albicans*. Not active at 5 mg per ml on *Trichophyton violaceum, Hormodendrum compactum, Sacch. cerevisiae, Cladosporium cucumerinum, Fusarium* sp., and *Verticillium dahliae*.

Reference: 1. British Patent 764,198, December 19, 1956.

Vinacetin

Produced by: Streptomyces sp. closely related to *S. albosporeus*.

Method of extraction: Broth adjusted to pH 5.0; filtered on paper to remove inert precipitate. Adsorption on cation exchange resin, elution with acetone. Evaporation of acetone, extraction of residual aqueous solution with ethyl acetate. Evaporation *in vacuo* of the ethyl acetate leaves a brown powder. The pure antibiotic can be crystallized from a chloroform-ether mixture.

Chemical and physical properties: Yellow platelet-shaped crystals; m.p. 157–158°C. Soluble in ethyl acetate, butyl acetate, methanol, and acetone. Slightly soluble in water. Insoluble in ether and petroleum ether. Soluble in alkaline water, with development of a violet color. More active and more stable at pH 5.0 than at pH 7.0 and 9.0. Negative ninhydrin, Sakaguchi, and Millon reactions. Positive Molisch, $FeCl_3$, and Fehling reactions.

Biological activity: Active against certain gram-positive bacteria (*Staph. citreus, Corynebacterium diphtheriae*) and mycobacteria. Inactive against gram-negative bacteria and fungi.

Toxicity: 5 mg per 15-gm mouse injected intravenously or intraperitoneally has no toxic effect; 10 mg kills the mice.

Reference: 1. Omachi, K. J. Antibiotics (Japan) **6A:** 73–79, 1953.

Violacetin

Produced by: Streptomyces sp. closely related to *S. purpeochromogenes*.

Method of extraction: Adsorption of antibiotic on diatomaceous earth at alkaline pH (8.0 to 9.0). Elution with 0.2 per cent HCl in acetone. Eluate adjusted to pH 6.8 to 7.0 and evaporated *in vacuo*. Brownish residue extracted with methanol. Methanol evaporated to leave crude violacetin. Crude violacetin purified further by chromatography on alumina. The column is loaded with an ethanolic solution of crude violacetin and eluted with 95 per cent ethanol. Violacetin can also be adsorbed on Amberlite IRC-50 and eluted with 2.0 per cent HCl in acetone (1).

Chemical and physical properties: Basic antibiotic. Can be precipitated out of ethanolic solutions as fine, light yellow, needle-shaped crystals. Violacetin hydrochloride does not melt at 210°C. Soluble in water, methanol, and ethanol, but insoluble or sparingly soluble in most other organic solvents. Violacetin is precipitated out of aqueous solutions by picric acid, Reinecke salt, and phosphotungstic acid. Negative biuret, Sakaguchi, glucosamine, xanthoproteic, Millon, Fehling, maltol, and $FeCl_3$ tests. Positive diazo and ninhydrin tests. H = 6.74%; C = 38.26%; N = 24.71%; Cl = 9.33% for the hydrochloride. No S present. Maximal light absorption in water at 293 mμ. Stable in acidic solutions; unstable at alkaline reaction (1).

Biological activity: Active against gram-positive

and gram-negative bacteria, including clostridia (2) and mycobacteria. Limited activity against *Pr. vulgaris*. Inactive against *Ps. aeruginosa*. Limited activity against certain fungi, such as *C. albicans*. Active against *D. pneumoniae*, *Sal. enteritidis*, and ornithosis virus in mice.

Toxicity: Administration of 0.6 mg intraperitoneally, 1.0 mg subcutaneously, 0.5 mg intravenously, or 5 mg orally does not produce any toxic symptoms in 13-gm mice. Larger doses produce delayed toxicity.

References:

1. Aiso, K. *et al.* J. Antibiotics (Japan) **8A:** 33–38, 1955.
2. Morita, Y. J. Antibiotics (Japan) **9A:** 226, 1956.

Violarin

Produced by: Streptomyces (*Actinomyces*) *violaceus* strains (1, 2).

Synonyms: Violarine I, Violarine 452-7. Similar to antivirubin.

Method of extraction: Broth extracted with chloroform at pH 6.9 to 7.5. Chloroform concentrated *in vacuo* to a thick syrup. Precipitates on addition of petroleum ether (b.p. 40–60°C). Purified by extraction with ether in a Soxhlet apparatus. Reprecipitated from benzene with petroleum ether (3).

Chemical and physical properties: Complex, containing several closely related substances. Bright red, amorphous substance with indicator properties. Decomposes above 130°C. Very soluble in benzene, dimethylformamide, formamide, chloroform, and hydrochloric, sulfuric, and acetic acids. Fairly soluble in ethanol and methanol; slightly soluble in carbon tetrachloride; insoluble in water and petroleum ether. Ultraviolet absorption spectrum maxima at 498 and 532 mμ (in n-butanol). $C_{22\text{-}24}H_{32\text{-}34}O_{8\text{-}9}$. Molecular weight, 419 to 445 (3).

Biological activity: Active *in vitro* against bacteria and vaccinia virus, and *in vivo* against influenza, vaccinia, tick encephalitis, and silkworm jaundice viruses (1, 3).

References:

1. Krassilnikov, N. A. *et al.* Antibiotiki 3(3): 18–22, 1958.
2. Solovieva, N. K. and Sorokina, E. I. Antibiotiki 3(4): 19–23, 1958.
3. Trachtenberg, D. M. *et al.* Abstr. Communs. Symposium on Antibiotics, Prague 194–196, 1959.

Viomycin

Produced by: Streptomyces puniceus (1), *S. floridae* (2), *S. vinaceus* (14) (This culture produced a purple substance very active on staphylococci and moderately active on mycobacteria), *S. californicus* (11), *S. abikoensum* (24), *S. olivoreticuli* (23), and *S. griseus* var. *purpureus*. The name viomycin was proposed for the first three producers listed here, and for other similar viomycin-forming organisms (20, 22).

Synonyms: Vinactin, vinactane (16), viocin, vionactia. Related to phthiomycin.

Method of extraction: I. Filtered broth adsorbed on IRC-50 (equilibrated at pH 7.5 with NaOH) at pH 7.5 and eluted with 0.35 *N* H_2SO_4 . Eluate neutralized with $Ba(OH)_2$ and the precipitated $BaSO_4$ filtered off. Filtrate adsorbed on XE-89 (equilibrated as above) at pH 7.5, and eluted and neutralized as above. Eluate decolorized with carbon. Addition of methanol crystallizes out viomycin sulfate (25). II. Adsorbed from broth on activated carbon. Eluted with dimethylcarbonate-methanol (3:7). Addition of diethyl ether gives crude precipitate. Also eluted from carbon with 0.1 *N* HCl in anhydrous methanol and precipitated on addition of petroleum ether. Purification by chromatography on alumina with 80 per cent methanol as solvent and developer. Active eluate freeze dried. Crystallized from warm absolute methanol (14).

Chemical and physical properties: Complex, containing two or three components which are strongly basic, possibly cyclic, polypeptides (10, 16). Mayer *et al.* (9) described the vinactin complex as containing three components, A, B and C. Viomycin is largely vinactin A (18), but there are no published reports confirming the presence of components identical to vinactins B and C in the broths of the viomycin-producers. *Viomycin base:* $C_{17\text{-}18}H_{31\text{-}35}N_9O_8$. *Sulfate:* Crystals; m.p. 280°C (decomposition) (hydrated form) (1) or 252°C (decomposition) (anhydrous) (2). Very soluble in water. Virtually insoluble in most common organic solvents (1). Ultraviolet absorption spectrum maximum at 268 mμ ($E^{1\%}_{1cm}$ 339) (0.1 *N* HCl) or at 280 to 282.5 mμ ($E^{1\%}_{1cm}$ 219) (0.1 *N* NaOH) (2, 23). Infrared spectrum given in references 14 and 23. $[\alpha]_D^{25} = -32°$ (c = 1 per cent in water) (1). Rotation varies with the pH of the solution (2). Positive Sakaguchi, Fehling (1), ninhydrin, and biuret tests (2). Negative Molisch, maltol, and Benedict tests (2, 8). Paper chromatography: Rf = 0.02 (80 per cent phenol) and 0.45 (n-butanol-glacial acetic acid-water, 4:2:1) (23). In aqueous solution (pH 5 to 6), has a half-life of 12 hours. Less stable at more acidic or basic pH (1). C = 37.19%; H = 5.86%; N = 20.61%; SO_4 = 17.63% (1); N = 2.7% (Van Slyke) (1) or 4.51% (after 2 hours) (2). *Hydrochloride:* Crystals; m.p. 248°C (decomposi-

tion) (14). Very soluble in water; slightly soluble in low molecular weight alcohols; virtually insoluble in other organic solvents (23). $[\alpha]_D = -33.5°$ (14). *Reineckate:* m.p. 210°C (decomposition, uncorrected) (23). *Picrate:* m.p. 215°C (decomposition) (14). *Oxalate:* Crystalline (2). Hydrolysis products include ammonia, urea, CO_2, and five ninhydrin-positive substances, including: L-serine, α,β-diaminopropionic acid, β-lysine, and a Sakaguchi-positive substance which gives a yellow *p*-hydroxyazobenzene *p*-sulfonic acid salt (needles; m.p. 212–215°C), with a pink reaction to the ninhydrin test (8, 13). Vinactin A was reported to yield lysine, serine, alanine, glycine, glutamic and aspartic acids on acid hydrolysis, and to contain a guanidino and creatinine group (10). Vinactin and viomycin both gave nine ninhydrin-positive substances, according to Arai (23). Vinactin B decomposes at 240°C; otherwise is the same qualitatively as A (10). Vinactin C acid hydrolysates contain serine, sarcosine, glycine, and another amino acid with an Rf close to aspartic and glutamic acids (10).

Biological activity: Active on mycobacteria. Very slightly active (20 to 81 μg per ml) on other bacteria and actinomycetes. Not active on yeasts, filamentous fungi, protozoa, or viruses (1–3). Bacteriostatic (4). Most active at alkaline pH. Activity reduced in the presence of serum. No cross-resistance with streptomycin. Resistance develops in a fashion similar to streptomycin (3). Active on tuberculosis in guinea pigs and mice (1, 4, 5). Active on *K. pneumoniae*, *Eberthella typhosa*, *Pr. vulgaris*, *B. anthracis*, *Pasteurella multocida*, and *Sal. gallinarum* infections in mice (4, 19). Active on *Mycobacterium leprae-murium* infections (mice) (15). Active *in ovo* on *Rickettsia typhi*. Active only at 100 μg per ml on intracellular *M. tuberculosis* (in macrophage), although active *in vitro* at 1.56 μg per ml (12).

Toxicity: Some discrepancy exists between the toxicities of viomycin and vinactin. *Viomycin sulfate:* LD_{50} (mice) 240 mg per kg intravenously (1), and 1381 mg per kg subcutaneously (6). LD_0 (mice) 7500 mg per kg orally (6). *Viomycin base:* LD_{50} (mice) 165 mg per kg intravenously (4). *Vinactin:* LD_{50} (mice) 35 mg per kg subcutaneously, 25 mg per kg intraperitoneally, and 4 mg per kg intravenously (16). Viomycin at 20 mg per ml causes degeneration of HeLa cells (21). Not locally irritating to animals (6), but produces painful nodules at the site of injection in human beings (7). Neurotoxic to cats; nephrotoxic to dogs (1, 6). Side effects in human beings include reversible eosinophilia, rashes, renal toxicity, electrolyte imbalance, possible impairment of vestibular function, and deafness (7).

Utilization: Very moderate effect on clinical tuberculosis, being less effective than streptomycin (7). Used only in cases in which the infecting organism is resistant to streptomycin and isoniazid, and with careful attention to toxic manifestations (17).

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23. Arai, T. *et al.* Antibiotics & Chemotherapy 7: 435–442, 1957.
24. Umezawa, H. Given in reference 23.
25. Friedman, I. J. *et al.* U. S. Patent 2,827,417, March 18, 1958.

Viomycin-like Antibiotic

Produced by: Streptomyces olivoreticuli (1).

Method of extraction: Broth-filtrate adsorbed on IRC-50 resin (Na^+ form). Eluted with 1.0 per cent H_2SO_4, pH of eluate adjusted to 6.0 to 6.4, precipitate which forms is filtered off, and filtrate concentrated *in vacuo*. Purified by fractionation with methanol, and salt conversion via the reineckate.

Chemical and physical properties: Sulfate: Very soluble in water. Insoluble in almost all organic solvents. Ultraviolet absorption spectrum maximum at 268 mμ ($E^{1\%}_{1cm}$ 284) in pH 7.0 buffer and in 0.1 *N* HCl, and at 280 mμ in 0.1 *N* NaOH. Infrared spectrum given in reference 1. Positive biuret, ninhydrin, Fehling, and Sakaguchi tests. Negative Benedict test. Rf = 0.2 (80 per cent phenol) and 0.45 (n-butanol-glacial acetic acid-water, 4:2:1). Hydrolysate gives pattern of ninhydrin-positive spots similar to viomycin hydrolysates. *Reineckate:* m.p. 210°C (decomposition, uncorrected).

Biological activity: Antimicrobial activity similar to viomycin, but somewhat less active.

Reference: 1. Arai, T. *et al.* Antibiotics & Chemotherapy 7: 435–442, 1957.

Virocidin

Produced by: Streptomyces flavoreticuli.

Synonyms: Probably identical with abikoviromycin and latumcidin.

Method of extraction: Broth-filtrate extracted with ethyl acetate, butyl acetate, ether, or chloroform. Ethyl acetate back-extracted into 0.1 *N* HCl. Aqueous extract extracted with ether at pH 7 to 8. Ether dehydrated, concentrated. Precipitated as the picrate. Recrystallized from carbon tetrachloride or carbon tetrachloride-chloroform.

Chemical and physical properties: Unstable substance, not capable of being crystallized as the base. *Picrate:* Yellow needles. No distinct melting point when heated slowly; turning brown at 120°C, gray above 200°C, and black at higher temperatures. Burns with an explosion when heated rapidly. Contains 47 per cent picric acid. Soluble in methanol, ethanol, acetone, chloroform, diethylene glycol, tetrahydrofuran, ethyl Cellosolve, and pyridine. Slightly soluble in water, benzene, ethyl acetate, carbon tetrachloride, ether, and dioxane. Insoluble in cyclohexane, petroleum ether, and ligroin. Ultraviolet absorption spectrum maxima at 238 ($E^{1\%}_{1cm}$ 390) and 335 mμ ($E^{1\%}_{1cm}$ 370) in water containing HCl, shifting to 250 ($E^{1\%}_{1cm}$ 370) and 335 mμ ($E^{1\%}_{1cm}$ 190) after 1 day. In water at pH 7.0, has a maximum at 243 mμ ($E^{1\%}_{1cm}$ 340), shifting to 241 ($E^{1\%}_{1cm}$ 200) and 282 mμ ($E^{1\%}_{1cm}$ 220) after 1 day. Negative $FeCl_3$, ninhydrin, biuret, Sakaguchi, sodium nitroprusside, Fehling, Molisch, Tollen, Nessler, Br_2, and $KMnO_4$ tests. Infrared spectrum given in reference 1. C = 49.61%; H = 3.55%; N = 10.95%. Becomes red-brown when inactivated.

Biological activity: Active in contact tests against rabies virus (assayed in mice). Very slightly active on bacteria: *E. coli* at 50 μg per ml and *Staph. aureus* at 250 μg per ml.

Toxicity: LD_{50} (mice) 12.5 mg per kg intravenously. Mice tolerate 0.5 mg per kg intracerebrally, and 25 mg per kg intradermally.

Reference: 1. Funaki, M. *et al.* J. Antibiotics (Japan) 11A: 138–142, 1958.

Virusin 1609

Produced by: Streptomyces lavendulae (4).

Chemical and physical properties: Basic substance. Yellow hygroscopic powder. Soluble in water, methanol, and ethanol. Insoluble in other organic solvents. Forms a picrate, helianthate, picrolonate, and hydrochloride. Stable at 3–5°C for 2 years as the crude powder. Acid-stable. Labile to alkali (1).

Biological activity: Active on gram-positive and gram-negative bacteria, mycobacteria, *Nocardia, Candida* (1), and viruses, including influenza A, A_1, and B *in vitro* and *in vivo* (2), and endemic encephalitis (1). Moderately active on vaccinia virus (3). Active on actinophage (1). Not active on Taylor's virus or staphylophage (1, 3). Active *in vivo* (animal not stated) on anthrax, tuberculosis, dysentery, diphtheria, brucellosis, and "strangles" of horses (1). Active on powdery mildew of the dog rose (5).

References:

1. Skriabin, G. K. Antibiotiki 2(1): 10–13, 1957.
2. Germanova, K. J. and Koroleva, V. G. Antibiotiki 2(1): 13–17, 1957.
3. Germanova, K. J. and Koroleva, V. G. Antibiotiki 2(3): 14–17, 1957.
4. Solovieva, N. K. and Delova, I. D. J. Microbiol. Epidemiol. Immunobiol. 29: 399–404, 1958.
5. Protsenko, E. P. *et al.* Given in Rautenshtein, Y. I. Mikrobiologiya (Engl. transl.) 28: 148, 1959.

Vulgarin

Produced by: Streptomyces sp. resembling *S. flavochromogenes.*

Method of extraction: Mycelium extracted with organic solvents.

Chemical and physical properties: White crystalline substance; m.p. 164–165°C. Soluble in various organic solvents. Insoluble in acidified water and petroleum ether. Positive $FeCl_3$ test. Ultraviolet absorption spectrum maxima at 227 and 338 mμ (solvent not stated). Infrared data given in reference 1.

Biological activity: Active on yeasts.

Reference: 1. Hosoya, S. *et al.* Japan. J. Bacteriol. **9,** 1954.

Xanthicin

Produced by: Streptomyces xanthochromogenes.

Method of extraction: Broth-filtrate extracted with ethyl acetate at acidic pH.

Chemical and physical properties: Fine yellow needles. Decomposes at 211–213°C. Ultraviolet absorption spectrum maximum at 270 mμ (ϵ = 35,235) (methanol) or at 260 (ϵ = 33,935) and 325 mμ (ϵ = 23,306) (in 0.1 *M* KOH). Infrared spectrum given in reference 2. $[\alpha]_D^{15} = +319°$ (c = 0.25 per cent in acetone). Positive Ehrlich aldehyde and $FeCl_3$ tests and tests for indole ring. Negative Fehling (hot) test. Methanolic solution forms a reddish orange precipitate with Brady's reagent. No reaction with phosphomolybdic acid or sodium molybdate. C = 57.80%; H = 5.70%; N = 5.19%. $C_{13}H_{15}NO_5$. Biological activity is reduced in alkali. Steam distillation with 40 per cent H_2SO_4 gives a monobasic, volatile acid, possibly acetic. Oxidation gives succinic acid. No amino, nitro, or carbonyl amide groups. May contain a δ-lactone ring.

Biological activity: Slightly active at 10 to 50 μg per ml on *Penicillium, Rhizopus, Aspergillus, Torula, Staph. aureus,* and *B. subtilis.* Inactive on *Sacch. sake,* gram-negative bacteria, and mycobacteria.

Toxicity: LD_{50} (mice) 105 mg per kg intraperitoneally.

References:

1. Arishima, M. *et al.* J. Agr. Chem. Soc. Japan **30:** 469–471, 1956.
2. Sekizawa, Y. and Miwa, K. J. Agr. Chem. Soc. Japan **30:** 471–474, 1956.

Xanthomycins

Produced by: Streptomyces sp. (1, 2, 8), *S. rutgersensis* (4), and *S. pseudogriseolus* (9).

Synonyms: Antibiotic H 1159 (4), protomycin, antibiotic 534 (9).

Method of extraction: I. Adsorbed from broth-filtrate at pH 7.5 to 8.0 on Norite containing 1 per cent Celite 545, and filtered. Filter cake washed with butanol-saturated water and eluted with 0.1 *N* HCl saturated with butanol. Eluate concentrated *in vacuo*, pH adjusted to 7.0 to 7.5, filtered, and extracted with chloroform. Back-extracted into water at pH 2.0. Aqueous extract concentrated *in vacuo* and antibiotics precipitated as picrates or helianthates. Purification by countercurrent distribution (chloroform-1 *M* phosphate buffer at pH 4.4) to give two major components, A and B, and a minor one, C. Component A precipitated as the reineckate and recrystallized from 95 per cent ethanol, then prepared as the hydrochloride (1). II. Extracted from broth-filtrate with ethylene dichloride at pH 7.0. Back-extracted into 0.01 *M* HCl, then into ethylene dichloride at pH 7.8, and then back into 0.01 *N* HCl. Process repeated. Freeze dried. Purified by countercurrent distribution (ethyl acetate-aqueous phosphate buffer, pH 6.2 to 6.3). Precipitated as the reineckate (2). Countercurrent distribution can also be carried out in a chloroform-0.5 *M* citrate buffer (pH 4.2) system. Active fractions extracted with chloroform and evaporated to dryness. Free base prepared by precipitation from chloroform with ether (6). III. Culture-broth adjusted to pH 4.0, filtered, then extracted with trichloroethylene at pH 8.5 to 9.5. Extract concentrated at 26°C, then back-extracted into water at pH 3.0 to 3.5. Aqueous extract extracted with chloroform at pH 6.0, then back-extracted into water at pH 2.0 to 2.5. Crystallized from 10 per cent water-propanol on addition of acetone. Purified by countercurrent distribution (trichloroethylene and 0.5 *M* sodium citrate at pH 6.0). Aqueous phases adjusted to pH 9.0 to 9.5. The organic phase washed with water at pH 9.0 to 9.5, then extracted with 0.25 *N* acetic acid (8, 10).

Chemical and physical properties: Basic substances, possibly containing a benzoquinone-like system (6). Complex contains three or four components (1, 2). Component A believed to be transformed into other active components under acidic conditions during purification (6). Rao and Peterson (6) reported that they had isolated the free base of A, but Dougall (10) showed that it was an irreversibly degraded mixture of products having little or no biological activity, containing a large amount of a biologically inactive neutral material, a small amount of ethanolamine, and a biologically active basic fragment which behaved like the

antibiotic on ionophoresis. The hydrochloride of A, however, was not inactivated during purification. *Xanthomycin A* (*i.e.*, inactive mixture): Deep red amorphous substance with a bitter taste. Ultraviolet absorption spectrum maxima (absolute ethanol) at 288 ($E^{1\%}_{1cm}$ 148) and 460 mμ ($E^{1\%}_{1cm}$ 118), and two inflections at 225 and 315 mμ (6). Infrared spectrum given in reference 6. Two weakly basic groups at pK 2.0 and 4.5 (6) or one at 4.8 (10). Evanescent purple color, changing to yellow in alcoholic sodium ethoxide. $C_{23}H_{29-31}N_3O_7$: C = 60.28%; H = 6.44%; N = 8.88% (6). *Xanthomycin A hydrochloride:* Bright orange-yellow rectangular plates (6) or crystalline red-yellow powder; m.p. 120–125°C (decomposition) (9). Very soluble in water and methanol (1). Acidic solutions are yellow; alkaline are deep red. Color change is reversible with loss of activity. On contact with air, the alkaline red solutions change irreversibly to yellow (6). Ultraviolet absorption spectrum maxima at 264 to 267 and 325 to 327 mμ (c = 4.5 mg per 100 ml of water) (1) or at 264.5 ($E^{1\%}_{1cm}$ 179.4) and 335 mμ ($E^{1\%}_{1cm}$ 18.8) (water, pH 2.0). In alkali, the ultraviolet spectrum is altered, but reverts to normal on re-acidification (2). In 0.1 *N* HCl, a maximum at 265 mμ, with a plateau at 340 to 380 mμ having a maximum at 345 mμ, was reported (6). Infrared spectrum given in references 6 and 10. $[\alpha]^{25}_{D} = +115°$ (c = 0.4 per cent in water) (6). pK = 3.0 and 7.3 (10). Negative Sakaguchi, ninhydrin, Molisch, $FeCl_3$, nitroprusside, acidified stannous chloride, fuchsin, cysteine, maltol, Millon, and Tollen tests (1, 9). Doubtful biuret test (9). Positive Benedict, Br_2 in CCl_4 , $KMnO_4$, silver nitrate, acidified KI sodium hydrosulfite, and periodic acid tests. Reduces alkaline ferricyanide (Park-Johnson). Slight precipitate with 2,4-dinitrophenylhydrazine and dimedone. Red solution in hydroxylamine. Can be reduced with loss of color and activity; color but not activity returns on shaking in oxygen. Destroyed by ultraviolet light (2, 3). Most stable at pH 1.0 at 100°C for 25 minutes (1). $C_{23}H_{29-31}N_3O_7 \cdot 2HCl$: C = 51.85%; H = 6.48%; N = 7.88%; Cl = 13.14% (6, 10). $C_{30}H_{40}N_4O_{11} \cdot 3HCl$: C = 48.54%; H = 5.06%; N = 7.84%; Cl = 13.86% (9). Contains one methoxyl, one methylimide, and two terminal methyl groups (6). *Reineckate:* Long orange needles; m.p. 165–170°C (decomposition) (1, 2) or 190–195°C (4). Very soluble in acetone. Soluble in warm methanol. Insoluble in cold water. $C_{38}H_{57}N_{12}O_{13}S_4Cr$: C = 42.68%; H = 5.4%; N = 15.43%; S = 12.1%. Molecular weight, 742 (1). Or $C_{29}H_{42}N_9O_7S_4Cr$: C = 43.22%; H = 5.28%; N = 15.49%; Cr_2O_3 = 10.77% (2). *Picrate:* Dark, reddish brown, amorphous powder. Decomposes at 117–120°C (9). *Sulfate:* m.p. 189–195°C (decomposition). *Xanthomycin B:* Not obtained in crystalline form. Hydrochloride is yellow and has similar solubility properties to those of A (1). Free B is more soluble in water than A and C. Yellow at acidic and pink at alkaline pH. Ultraviolet absorption spectrum maxima at 290 and 330 mμ (pH 1.0 or 6.0). At pH 11.0, has a single peak at 280 mμ. Reduces alkaline ferricyanide. *Xanthomycin C:* Relatively inactive substance occurring in minor amounts and believed related to A and B. Less water-soluble than A or B. Has a single peak at 265 to 275 mμ at pH 1.0 to 6.0, and a single peak at 280 mμ at pH 11.0 (3). Acid hydrolysis of A gives ethanolamine, methylamine (7, 8, 10), ammonia, and a large amount of dark brown humin-like material (7, 8). Tetrahydroxanthomycin A can be methylated with simultaneous hydrogenation to give a methyl ether. Methyl ether can be oxidized in neutral $KMnO_4$, acidified, and extracted with ether. Ether-insoluble fraction contains *xanthomycinic acid I*, $C_7H_{11}N_3O_{10}$, colorless prisms, m.p. 147–148°C, with primary amide and carboxyl groups; and *xanthomycinic acid II*, $C_4H_3O_3$, long colorless needles, m.p. 118–119°C (7).

Biological activity: Very active on gram-positive and gram-negative bacteria (1, 2). Not active on yeasts or fungi (5). Active in contact tests (in mice) on *Toxoplasma gondii* (9).

Toxicity: LD_{50} (mice) 160 μg per kg (1) or 100 μg per kg (2) intravenously.

References:

1. Thorne, C. B. and Peterson, W. H. J. Biol. Chem. **176:** 413–428, 1948.
2. Mold, J. D. and Bartz, Q. R. J. Am. Chem. Soc. **72:** 1847–1849, 1950.
3. Rao, K. V. and Peterson, W. H. 12th Intern. Congr. Pure Appl. Chem. 282–283, 1951.
4. Hosoya, S. *et al.* J. Antibiotics (Japan) **4:** 467–469, 1951.
5. Hosoya, S. *et al.* Japan. J. Exptl. Med. **22:** 135–138, 1952.
6. Rao, K. V. and Peterson, W. H. J. Am. Chem. Soc. **76:** 1335–1340, 1954.
7. Rao, K. V. *et al.* J. Am. Chem. Soc. **77:** 4327–4330, 1955.
8. Dougall, D. K. and Abraham, E. P. Nature, London **176:** 265, 1955.
9. Okami, Y. *et al.* J. Antibiotics (Japan) **8A**: 126–131, 1955.
10. Dougall, D. K. J. Chem. Soc. 628–633, 1957.

Xanthothricin

Produced by: Streptomyces sp. closely related to *S. albus.*

Method of extraction: Extraction of culture-filtrates with chloroform, counterextraction of the chloroform solution with sodium acid sulfite solution, oxidation of the $NaHSO_3$ extract with hydrogen peroxide, re-extraction with chloroform, followed by crystallization of the antibiotic from chloroform-ether solution.

Chemical and physical properties: Yellow needle-like crystals; m.p. 165°C. Soluble in water, chloroform, ethyl acetate, and ethanol. C = 43.64%; H = 3.82%; N = 35.21%; O = 17.34%. No P, S, or halogens detected. Maximal light absorption at 257.5 and 390 to 402 mμ in phosphate buffer at pH 6.0. Inactivated by prolonged standing at low or high pH values. Xanthothricin loses its color at pH 10.5.

Biological activity: Moderate activity against certain gram-positive and gram-negative bacteria and mycobacteria. No activity against fungi.

Toxicity: LD_{50} (mice) 1.7 mg per kg intravenously, 8.4 mg per kg orally.

Reference: 1. Machlowitz, R. A. *et al.* Antibiotics & Chemotherapy **4**: 259–261, 1954.

Zaomycin

Produced by: Streptomyces zaomyceticus.

Method of extraction: Extraction at pH 2.0 from broth-filtrate in n-butanol. Back-extraction into water at pH 8.5. This process repeated, then the aqueous solution concentrated *in vacuo*. The crude powder dissolved in methanol and precipitated by ether. Further purification carried out by chromatography on alumina. The column is loaded with ethanol and developed by aqueous ethanol and water. Purified zaomycin sodium salt is obtained by this method. Free zaomycin acid precipitates out of water solutions at pH 3.5. Crystallization obtained from an ethyl acetate-methanol mixture.

Chemical and physical properties: Acidic substance, pale yellow; m.p. 242–246°C (decomposition). Unstable. Maximal stability in water at pH 7.0 to 8.0. Slightly soluble in water. Soluble in dilute acids and alkalies and in methanol and ethanol. Insoluble in most organic solvents. Ninhydrin, Millon, biuret, and $FeCl_3$ (reddish brown color) tests positive. Negative Fehling and Liebermann nitroso reactions.

Biological activity: Active against gram-positive bacteria. Inactive against gram-negative bacteria and fungi. Activity against mycobacteria limited to certain nonpathogenic strains. Static action on *Trichomonas foetus*, but inactive on *Trichophyton interdigitale.*

Toxicity: Intravenous injection of 301 μg per kg of sodium zaomycin in mice is nontoxic.

Reference: 1. Hinuma, Y. J. Antibiotics (Japan) **7A**: 134–136, 1954.

Appendix

List of Additional Antibiotics and Antibiotic Preparations

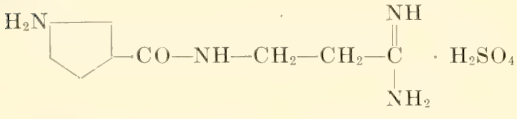
A number of other antibiotics and antibiotic preparations are described in the literature, most of which appeared while this compilation was being completed. They are briefly listed here.

2-Acetyl-2-decarboxamide-oxytetracycline: Oxytetracycline derivative. Produced by *S. rimosus*. Its antibiotic spectrum is similar to oxytetracycline, but is less active. (Hochstein, F. A. *et al.* J. Am. Chem. Soc. **82:** 5934–5937, 1960.)

Actiphenol: A biologically inactive glutarimide produced by cycloheximide-nonactin-producing strains of *Streptomyces*. $C_{15}H_{17}O_4N$; m.p. 199–200°C. Ultraviolet maxima at 215, 264, and 354 mμ in methanol. (Highet, R. J. *et al.* Helv. Chim. Acta **42:** 1523–1526, 1959.)

Akitamycin: An antifungal tetraene. (Soeda, M. *et al.* J. Antibiotics (Japan) **12B:** 293–299, 1959.)

Amidinomycin: A basic antibiotic, produced by a *Streptomyces*; active against gram-positive bacteria. The sulfate in aqueous solutions absorbs light at 211 to 212 mμ. Structure of the sulfate:



(Nakamura, S. *et al.* J. Antibiotics (Japan) **13A:** 362–365, 1960.)

Amminosidin: An antibiotic of the neomycin-catenulin-kanamycin type, produced by *S. crestomyceticus*. Synonyms: crestomycin, antibiotic 1600 F.I., antibiotic F.I. 5853, formiglucine. (Arcamone, F. *et al.* Giorn. microbiol. **7:** 251–272, 1959; Calandi, C. and Bartolozzi, G. Riv. clin. pediat. **64:** 269–284, 1959.)

Amycin: A basic water soluble antibiotic produced by a strain of *S. lavendulae*. Active against bacteria resistant to streptomycin, neomycin, viomycin, and streptothricin. Negative Sakaguchi and positive ninhydrin reactions. LD_{50} (mice) = 120 mg per kg intraperitoneally. (Craveri, R. Ann. Microbiol. Enzimol. **10:** 115–121, 1960.)

Amylocyanin: A litmus-like pigment produced by *Streptomyces coelicolor*. Has antifungal properties. (Müller, R. Centr. Bakteriol. Parasitenk., Abt. I Orig. **46:** 195–212, 1908.)

Antibiotic 4: Basic antibiotic produced by *S. griseoflavus*; about 2.5 times more toxic than streptomycin. (Heki, M. *et al.* Japan. Med. J. **3:** 41–47, 1950.)

Antibiotic A.E. 56: An antifungal heptaene, closely related to ayfactin and produced by a species of *Streptomyces*. (Staron, T. and Faivre-Amiot, A. Compt. rend. **250:** 1730–1732, 1960.)

Antibiotic H 18: Basic antibiotic produced by a chromogenic *Streptomyces* species. Delayed toxicity. Reineckate; m.p. 181–182°C. (Hosoya, S. *et al.* J. Antibiotics (Japan) **2:** 550, 1948.)

Antibiotic of Vernon and Dye: An antibiotic with a broad antibacterial spectrum, produced by a white-spored *Streptomyces*. (Vernon, T. R. and Dye, M. H. New Zealand J. Agr. Research **2:** 982–989, 1959.)

Antibiotic P 9: An antibiotic produced by a *Streptomyces*. It is nonphytotoxic and is translocated in plants. Active *in vivo* against certain fungi. (Davis, D. *et al.* Phytopathology **50:** 841–843, 1960.)

Antibiotic PA 96: Chemically stable antiviral substance. (Ambrus, J. L. *et al.* Antibiotics & Chemotherapy **3:** 16–22, 1953; Chinn, B. D. Proc. Soc. Exptl. Biol. Med. **80:** 359–360, 1952.)

Antibiotic complex of Sgarzi et al.: An antibiotic active against gram-positive bacteria. Hydrolysate yields amino acids. Ultraviolet light-absorption maximum at 282 mμ. (Sgarzi, B. *et al.* Nature, London **187:** 1029–1030, 1960.)

Antibiotic of Raya: A water soluble antibiotic, produced by a *Streptomyces*. Active against bacteria, mainly *Brucella* and fungi. (Raya, J. M. Rev. farm. (Buenos Aires) **101:** 151–157, 1959; Chem. Abstr. **55:** 1789g, 1961.)

Antibiotics M-II and 719: Produced by strains of *Streptomyces violaceus*. Dark red substances soluble in ethyl alcohol. Can partially inactivate

influenza viruses *in vitro*. Effective in suppressing influenza viruses (A, A_1, and A_2) in chick embryo. (Furer, N. M. Antibiotiki **4**(3): 30–36, 1959.)

Antibiotics with pH indicator properties: In the following papers, poorly described antibiotics with litmus-like properties have been discussed: Sanchez-Marroquin, A. and Zapata, M. Appl. Microbiol. **2:** 102–107, 1954; Conn, J. E. J. Bacteriol. **46:** 133–149, 1943; Oxford, A. E. J. Bacteriol. **51:** 267–269, 1946; Kriss, A. E. Compt. rend. acad. sci. U.R.S.S. **4:** 283–287, 1936; Frampton, V. L. and Taylor, C. F. Phytopathology **28:** 7, 1938.

Antifungal antibiotics 2814P and 2814H: Two polyenic antifungal antibiotics produced by a strain of *Streptomyces*. P is a pentaene and H is a heptaene. (Thrum, H. *et al.* Naturwissenschaften **47:** 474, 1960.)

Antifungal antibiotic S 39: A water-soluble antifungal substance produced by strains of *Streptomyces* absorbing light at 260 mμ. (Gopalkrishnan, K. S. *et al.* Hindustan Antibiotics **2:** 7, 1959.)

Antitumor antibiotic NSC A-649: An antitumor antibiotic with activity against gram-positive bacteria. Chemically the substance has properties similar to the magnesium salt of aureolic acid, antibiotic M5-18903, aburamycin, and antibiotic L A 7017. (Schmitz, H. *et al.* Antibiotics & Chemotherapy **10:** 740–746, 1960.)

Ayamycins: A mixture of antitumor substances produced by a *Streptomyces*. One of these components, ayamycin A_2, is an orange-yellowish substance which absorbs light at 220, 320, and 430 mμ in methanol. It has slight activity against gram-positive bacteria. (Sato, K. J. Antibiotics (Japan) **13A:** 321–326, 1960; Tanno, K. J. Antibiotics (Japan) **13A:** 391–400, 1960.)

Bacteriolytic factors: Produced by *Streptomyces albus*. (McCarthy, M. J. Exptl. Med. **96:** 555–580, 1952; Floch, H. Compt. rend. soc. biol. **143:** 449–450, 1949.)

Capacidin: A pentaenic antifungal antibiotic produced by a strain of *Streptomyces*. (Brown, R. and Hazen, E. L. Antibiotics & Chemotherapy **10:** 702–708, 1960.)

Carzinostatin: An antitumor substance produced by a *Streptomyces* related to *S. albus*. It is composed of two fractions, one soluble in methanol and one insoluble in methanol, which have a synergistic action against *Sarcina lutea*. (Shoji, J. J. Antibiotics (Japan) **14A:** 27–33, 1961.)

Cinerubins: Antitumor substances similar to rhodomycin, produced by various strains of *Streptomyces*. (Corbaz, R. *et al.* Arch. Mikrobiol. **25:** 325–332, 1957; Loustalot, P. *et al.* Ann. N. Y. Acad. Sci. **76:** 838–848, 1958; Ettlinger, L. *et al.* Chem. Ber. **92:** 1867–1879, 1959.)

Datemycin: A narrow spectrum antifungal antibiotic (active against some strains of *Candida*) probably produced by an actinomycete. Ultraviolet maxima at 235 and 247 mμ. (Kuroya, M. and Koyama, Y. Japanese Patent 6648, July 31, 1959, cited in Chem. Abstr. **54:** 832f, 1960.)

Dihydro E 73: A chemical derivative of antitumor substance E 73. Has some activity in certain cancer patients. (Wright, J. C. *et al.* Cancer Chemotherapy Repts. **8:** 7–17, 1960.)

Emimycin: An antibiotic with poor activity against bacteria and fungi. Produced by a strain of *Streptomyces* similar to *S. griseochromogenes*. Water soluble, U.V. max. (methanol) at 222–223, 276 and 330–332 mμ. (Terao, M. *et al.* J. Antibiotics (Japan) **13A:** 401–405, 1960.)

Erygrisin: A water soluble pH indicator antibiotic which is mainly active against gram-positive bacteria. LD_{50} (mice) = 50 mg per kg subcutaneously. Produced by *Streptomyces erythrogriseus*. (Lyra, F. D. A. *et al.* Ref. Inst Antibioticos, Pernambuco **2:** 99–107, 1959.)

Eurotin: An antifungal antibiotic produced by *S. griseus*. Shows weak light-absorption maxima at 365 and 385 to 390 mμ in aqueous solutions. (Hosoya, S. Tôhoku Igaku Zassi **6:** 285–292, 1959.)

Ferromycin: A new antibiotic produced by *S. myanaensis*. Called ferromycin because ferrous sulfate is needed for production. Basic antibiotic, decomposes at 188°C, $C_{21}H_{54}O_{14}N_7.2HCl$ hydrochloride. Active against gram-positive and gram-negative bacteria. (Taguchi, H. and Yoshikawa, K. J. Ferm. Technol. **39:** 44–52, 1961.)

Frenolicin: An antibiotic containing no nitrogen, and having very little activity against bacteria and fungi. Produced by *S. fradiae*. (Van Meter, J. C. *et al.* Conf. on antimicrobial agents, Washington, D. C., 1960.)

Geminimycin: An antibiotic composed of two biologically inactive components. Mixture of the two components is active against gram-positive bacteria. (Rao, K. V. *et al.* Conf. on antimicrobial agents, Washington, D. C., 1960.)

Heptamycin: A heptaenic antifungal antibiotic produced by a *Streptomyces*. (Henis, Y. *et al.* J. Gen. Microbiol. **23:** 345–355, 1960.)

HON: A water soluble nontoxic antimycobacterial antibiotic produced by *Streptomyces akiyoshiensis*. U.V. max. at 271 mμ. $C_5H_9O_4N$ (δ-hydroxy-γ-oxo-L-norvaline). (Kanazawa, K. *et al.* Am. Rev. Resp. Dis. **81:** 924, 1960; Tatsuoka, S. *et al.* J. Antibiotics (Japan) **14A:** 39–43, 1961.)

Imoticidin: An antibiotic produced by strains of *S. albus*. Active against gram-positive bacteria and fungi. (Inouye, T. *et al.* Ber. Ōhara Inst. landwirtsch. Biol. Okayama Univ. **11:** 95–131, 1959; Nôgaku Kenkyû **46:** 120–128, 1959.)

Lankamycin and Lankacidin: Two neutral, colorless, weakly antibacterial substances produced by a strain of *S. violaceoniger*. (Gäumann, R. *et al.* Helv. Chim. Acta **43:** 601–606, 1960.)

Marinamycin: Weak activity against gram-positive bacteria and fungi. (Soeda, M. *et al.* J. Antibiotics (Japan) **12B:** 300–304, 1959.)

Minomycin: Orange antibiotic produced by a *Streptomyces*; pH indicator. Active against gram-positive bacteria and certain tumors. Light-absorption maxima at 241, 277, and 444 mμ in methanol. (Nishimura, H. *et al.* J. Antibiotics (Japan) **13A:** 327–334, 1960.)

Mithramycin: A toxic antitumor antibiotic. Synonyms: antibiotics PA 144 and NSCA 2371. (Curreri, A. R. *et al.* Cancer Chemotherapy Repts. **8:** 18–22, 1960; Parker, G. W. *et al.* Cancer Chemotherapy Repts. **8:** 23–26, 1960.)

Monomycin: An antibiotic of the neomycin group produced by *Streptomyces circulatus* var. *monomycini*. (Gause, G. F. *et al.* Antibiotiki **5**(**4**)**:** 3–37, 1960.)

Porfiromycin: An antibacterial, purple substance produced by *Streptomyces ardus*. (Deboer, C. *et al.* Conf. on antimicrobial agents, Washington, D. C., 1960.)

Primocardin: A neutral antitumor substance with a modest amount of antibacterial activity, produced by *Nocardia ukayae*. Water soluble. U.V. max. (0.05 *N* HCl) 253 mμ. $C_8H_{12}N_2O_3$. (Sumiki, Y. *et al.* J. Antibiotics (Japan) **13A:** 416, 1960.)

Pyrromycin: A red antibiotic produced by a *Streptomyces*. Active against gram-positive bacteria. In chloroform, light-absorption maxima at 498, 518, and 533 mμ, with inflections at 395, 415, 469, and 482 mμ. Related to rhodomycin. (Brockmann, H. *et al.* Chem. Ber. **92:** 1904–1909, 1959.)

9-β-D-Ribofuranosylpurine: This nucleoside had previously been found in *Agaricus neburaris*; is also found to be produced by a *Streptomyces*. It is moderately active against some mycobacteria and fungi. (Isono, K. and Suzuki, S. J. Antibiotics (Japan) **13A:** 270–272, 1960.)

Teruchiomycin: A macrolide antibacterial substance ($C_{28}H_{43}NO_{16}$) produced by *S. eurocidicus*. (Umezawa, H. *et al.* Japanese Patent 850, February 13, 1958; cited in Chem. Abstr. **52:** 20921, 1958.)

Teleocidin: Produced by a variant of *S. mediocidicus*. Extracted from mycelium by ethanol in form of a white crystalline powder. Highly toxic to various aquatic organisms, notably Japanese killifish (*Coryzias latipes*) and mice; no inhibiting effect upon the growth of microorganisms. (Takashima, M. and Sakai, H. Bull. Agr. Chem. Soc. Japan **24:** 647–651, 652–655, 1960.)

Theiomycetin: An antibiotic produced by a *Streptomyces*, active against various bacteria and fungi. It contains sulfur, and melts at 230°C. (Shibata, M. Takeda Kenkyusho Nempo **18:**44-48, 1959, cited in Chem. Abstr. **54:** 19840c, 1960.)

Toyamycin: An antifungal tetraene. (Ishibashi, H. 104th Japan. Conf. on Antibiotics, May, 1957.)

Tundromycin: A chemically unstable antibiotic produced by *S. globisporus tundromycini*. It is characterized by a narrow spectrum, and is active against strains of staphylococci and sarcinae. (Kovalenkova, V. Bull. Moscow Soc. Naturalists, Ser. Biol. **62**(**2**)**:** 79, 1957.)

Tylosin: A weakly basic substance soluble in organic solvents. Produced by a strain of *S. fradiae*. Acid hydrolysis yields mycarose and an antimicrobial compound, desmycosin. Active against viruses, gram-positive and gram-negative bacteria. (Abstr. 135th Meeting Am. Chem. Soc. 16C, 1959.)

Virtosin: An antifungal antibiotic produced by *Streptomyces olivochromogenes*. Positive Sakaguchi, negative ninhydrin reaction. (Miyake, A. *et al.* Japanese Patent 6149, August 10, 1957, cited in Chem. Abstr. **52:** 20919b, 1958.)

Yunamycin: An antifungal antibiotic (probably a tetraene). Ultraviolet absorption spectrum maxima at 290, 304, and 320 mμ. Produced by *Streptomyces fungicidicus*. (Umezawa, H. and Nagase, M. Japanese Patent 3392, May 7, 1959, cited in Chem. Abstr. **53:** 20708d, 1959.)

Zygomycin A: A paromomycin-like antibiotic. (Kanazawa, K. *et al.* Am. Rev. Resp. Dis. **81:** 924, 1960.)

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