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REVIEW ARTICLE

THE CHEMICAL ESTIMATION OF DIGITALIS AND STROPHANTHUS GLYCOSIDES

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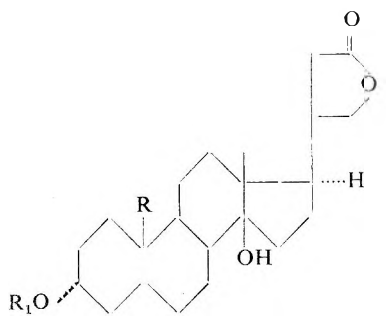
Certain glycosides of plant origin possess valuable cardiotonic properties. The Families Scrophulariaceae and Apocynaceae are the plant orders which contain most members furnishing drugs of therapeutic value. If in this connection the toad poisons are included, the heart-active substances from a chemical viewpoint may be subdivided into three large groups: the digitalis-strophanthus group, the scilla-helleborus group and the nitrogenous venoms secreted by the parotid glands of toads. The chief differences between the groups are: the members of the first group are glycosides, the aglucones of which contain a five-membered lactone ring, the members of the second group are glycosides, the aglucones of which contain a six-membered lactone ring and the members of the last group are suberylarginine derivatives of acetylated hydroxylactones more closely related to the scilla group than to the digitalis group.

Only the first group will be discussed here. The ring system of the cardiac glycosides from the genera *Digitalis* and *Strophanthus* is given below: —



The sugar moiety R_1 may be composed of as many as four sugar molecules. The sugars are for the most part α-desoxy sugars (cymarose, digitoxose and sarmentose) and digitalose, glucose and rhamnose. When an α-desoxy sugar is joined directly to the nucleus, the hydrolysis of the glycoside bond is easily effected; when rhamnose or glucose is the first sugar molecule the union is much firmer, and drastic conditions are necessary for splitting off the sugars. Digitoxin may be mentioned as an example of the first group; ouabain as an example of the second group. In fact, it is only very recently that Mannich has succeeded in

splitting off rhamnose from ouabain without dehydrating the aglucone at the same time. The aglucones are steroids* with a $\Delta^{\alpha:\beta}$-butenolide C_{17} side chain and they are hydroxylated in positions 3 and 14. Many of the aglucones have additional hydroxyl groups in positions 5, 12 and 16. Summarising, it may be said that the characteristic cardiac-stimulating activity of the glycosides is due to the steroid skeleton of the aglucone, the C_{17} side chain, the C_{14} hydroxyl group and to a minor extent to the structure of the sugar moiety. The glycosides are present in the plants in mixtures. From *Digitalis purpurea* a number of substances have been isolated: the purpurea glycosides, which on partial hydrolysis give digitoxin and gitoxin, gitalin, the inactive glycoside diginin, saponins such as digitonin, tannins and enzymes.

It is about fifty years since the first paper was published on the biological assay of digitalis, and there is still controversy among the biologists as to which method shall be used. In recent publications Gold *et al*[1] advocate a human assay of digitalis to overcome the discrepancy between the potencies of digitalis preparations obtained by the cat method and the actual activity in man, especially by oral administration. This discrepancy has emphasised the necessity of finding a more specific method of analysis than the crude lethal dose method. Thus it is desirable that any assay method of cardiotonic drugs should take account of not only the amount and the distribution of the active glycosides, but also of the absorbability of the active principles from the intestine.

Chemical Testing

It is symptomatic that in the last few years some very interesting papers on the chemical estimation of digitalis and strophanthus preparations have been published. Some of these papers will now be reviewed and commented upon.

The chemical assay of digitalis leaf is a complicated matter. The ideal method should give figures for:—(1) the total amount of active glycosides; (2) the amount of each different glycoside; (3) the amount of aglycones; (4) the absorbability of the glycosides. As the potency of the glycosides varies with the number of sugar molecules attached to the nucleus it is also desirable to have information of the amount of sugars attached.

The structure of the compounds also gives some clue to the solution of the problem. Purely physical methods such as ultra-violet and infra-red spectroscopy would give some information. Colorimetric methods which determine either the sugars or the butenolide group should give reliable figures of the amount of active principles present, but unfortunately no method is known, which differentiates between the glycosides and the aglucones when working on the genin part of the glycoside. However, by combination of a method based on the sugars and a method

* The alkaloids from *Erythrophleum* have cardiotonic activity. They are not steroids.

based on the butenolide group it is easy to calculate the amounts of glycosides and aglucones present.

It should also be possible to separate the glycosides from the aglucones by chromatography on aluminium oxide, or charcoal, or paper strips or by partition chromatography. The separation of the different glycosides (or, after hydrolysis, the aglucones) would also be possible by some kind of chromatography. The chromatographic spectrum of the glycosides could be determined by the aid of pure glycosides.

The extraction of the glycosides from digitalis leaf powder or from galenicals can be done in different ways. Usually an aqueous, methyl alcoholic or ethyl alcoholic extract is prepared. The tannins are removed by shaking with fresh lead oxide or precipitated by lead acetate solution, the precipitate is filtered off and the dissolved lead removed by hydrogen sulphide or sodium phosphate. From this partially purified extract the glycosides and aglucones are extracted with chloroform. Free sugars are left in the aqueous phase. When the final estimation is made on the sugar moiety of the glycosides the latter are hydrolysed and the free sugars estimated.

This route of isolation and purification has some disadvantages, as at all stages large losses are suffered. Extraction with water or with alcohols of different strengths may result in a fractionation of the glycoside complex, as the different glycosides have very different solubilities in these solvents. When the tannins are precipitated by lead acetate, coprecipitation of the glycosides will occur, and it is difficult to wash the precipitate free from them. The extraction of the glycosides and the aglucones from the aqueous or alcoholic solution by chloroform is a very troublesome procedure. The presence of saponins enhances the solubilities of the active principles in the aqueous phase, and even repeated extraction will not give a quantitative yield.

Methylene chloride is a much better solvent than chloroform when extracting pure or purified glycosides from tablets containing lactose because the solubility of lactose in methylene chloride is much less than in chloroform. Lactose disturbs most of the known colour reactions, especially those carried out in alkaline solution, and measuring the transmission at about 500 mμ is unreliable when chloroform is used as the solvent.

Methods Dependent on the Butenolide Side Chain

All glycosides from the digitalis and strophanthus groups show in ethyl alcoholic solution an absorption maximum between 215 and 220 mμ in most cases at 217 mμ with log ε about 4·2. This absorption is very characteristic and is due to the $\Delta^{\alpha:\beta}$-butenolide group. Of course, it is not possible to differentiate between glycosides and aglucones. The method has been used for some years in this laboratory as routine control on injections containing ouabain or digitoxin. When suitable

equipment is available and interfering substances are known to be absent, the method is very rapid.

Extinction plotted against amount of glycoside present gives a straight line when the measurements are made on the peak of the absorption curve. If alkali is added, it is possible to follow the isomerisation of the glycosides as the peak at 217 mμ gradually disappears, which is a good identification of the substance present.

The following methods depend on a reaction with the butenolide group: the Legal reaction with sodium nitroprusside and alkali, the Knudson and Dresbach method with alkaline sodium picrate solution, the Raymond method with *m*-dinitrobenzene in alkaline solution, the Kedde method with alkaline sodium 3 : 5-dinitrobenzoate solution and the Warren, Howland and Green method with sodium β-naphthoquinone-4-sulphonate.

The Legal reaction depends on the formation of a red colour when sodium nitroprusside is added to an alkaline solution of the glycosides. The colour test has been described by Jacobs, Hoffman and Gutus[2] and has been extensively used in research work on the cardiac glycosides. Qualitatively the test is performed so that a relatively large amount (0·01 g.) of the glycoside to be tested is dissolved in pyridine and an equal volume of water is added. A few drops of a 10 per cent. sodium hydroxide solution are added and then 1 ml. of 0·3 per cent. sodium nitroprusside solution. When a heart-active glycoside is present, a bright red colour develops, which slowly fades. The test has been criticised by Elderfield[3], who proposed the use of potassium ferricyanide instead of sodium nitroprusside. Hardegger, Heusser and Blank[4] have shown that the Legal test is not specific for the butenolide group, but that certain related synthetic products also give a positive reaction. The reaction probably involves a condensation of the butenolide group with the reactive NO group (in alkaline solution), and the colour is probably due to salt formation of an isonitroso derivative.

Kedde[5] has developed a quantitative method for the assay of digitalis preparations based on the Legal reaction. He works in a buffered solution of *p*H 11, and obtains a relatively stable colour. The absorption band has a maximum at about 470 mμ.

Some years ago Knudson and Dresbach[6] used the Baljet[7] reaction to estimate digitalis preparations. The Baljet reaction is based on the Jaffe reaction[8]: creatinine gives with alkaline picrate solution a red colour. The glycosides give a red-orange colour when their solutions are treated with alkaline picrate solution. The method has recently been of current interest in the United States of America and Bell and Krantz[9] especially have contributed some interesting investigations. In a series of papers they have shown that the method gives reliable results when the transmission is measured by means of a photoelectric colorimeter. In a collaborative study[10] of the assay of digitalis and its preparations by the Baljet reaction and the cat method it was shown that significant correlation between the two methods existed, showing that both methods

measure the same activity of the drugs. Their method has been made the basis for method I, Digitoxin Colorimetric Controls, in the U.S.P. XIII. For the decolorisation of ethyl alcoholic extracts of digitalis leaf powder they use lead acetate and sodium phosphate. No extraction of the glycosides with chloroform is used, but the reagent, alkaline picrate solution, is added directly to the purified aqueous extract of the drug. After 20 minutes the difference in transmission between the extract and a blank is measured at about 525 mμ. As standards the U.S.P. digitalis reference standard tincture and the same tincture of half strength are used.

U.S.P. XIII uses absolute methyl alcohol to extract digitoxin from tablets.[11] It is necessary to change the composition of the reagent to avoid precipitation of digitoxin and sodium picrate in the final solution. To the methyl alcoholic digitoxin solution containing 0·1 to 1·0 mg. in 10 ml. are added 10 ml. of reagent (2 g. of trinitrophenol dissolved in methyl alcohol to 50 ml., 5 ml. of sodium hydroxide solution (1 in 10) and water to 100 ml.) and after 30 minutes the transmission is measured at about 525 mμ.

The modified Knudson-Dresbach chemical assay has been criticised from different aspects and published results indicate that the values obtained are generally higher than the values obtained by bioassay procedures. The most serious drawback is that glycosides as well as aglucones are determined and reported, as Baljet pointed out. Goldstein[12] has shown that ageing of digitalis tinctures does not apparently affect their potencies according to the chemical assay, which is rather improbable. Elmqvist and Liljestrand,[13] working with infusions and pills, point out that while the bioassays (guinea-pig) indicate a rapid deterioration of the preparation with time, the potencies obtained by the chemical assays are constant or sometimes even increase. Vos and Welsh[14] have reported similar results.

Another disadvantage of the Knudson-Dresbach method is that the difference between the absorption curve of the picrate ion in alkaline solution and the absorption curve of the test solution with alkaline picrate solution is very small, while difficulties of purely technical kinds are present. The Lambert-Beer law is not fulfilled. Lactose interferes as it gives a pronounced colour with the alkaline reagent. An advantage is that the curves obtained by plotting photometer readings against time indicate the presence of interfering substances when the resulting curves differ in shape from those obtained with a standard.

In 1935, Marthoud[15] pointed out that a solution of *m*-dinitrobenzene and heart-active glycosides in ethyl alcohol, on addition of alkali, develops a bright blue-violet colour. This colour test was quantitatively used by Raymond[16] to estimate the amount of ouabain in East African arrow poisons prepared from seeds of *Strophanthus* species and from *Acocanthera* wood. Rasmussen[17] used the method to estimate ouabain in injections, Anderson and Chen[18] to assay digitoxin. Canbäck modified the method and estimated digitalis, strophanthus[19] and uzara

preparations[20]. The reaction is a special case of the general reaction of *m*-dinitrobenzene with active methylene groups in alkaline solution.

Anderson and Chen[18] use the following technique: 150 to 250 μg. of digitoxin is dissolved in 10 ml. of 47·5 per cent. ethyl alcohol and 1 ml. of a 1 per cent. *m*-dinitrobenzene solution in absolute ethyl alcohol is added. The mixture is placed in an ice-bath for 5 to 10 minutes. Then 2 ml. of a 20 per cent. solution of sodium hydroxide is added and the mixture returned to the ice-bath. Exactly 5 minutes after the addition of the sodium hydroxide the transmission is measured in a photoelectric colorimeter equipped with an orange filter. Water is used as a blank. The amount of digitoxin present is read from a standard curve. The colour fades fairly rapidly and it is necessary to read exactly 5 minutes after the addition of the alkali. The colour intensity is dependent on the alcoholic strength and the temperature, both of which must be rigidly controlled.

To overcome most of the difficulties the following technique[19] for the estimation of digitoxin tablets has been used in this laboratory: —

As much of well powdered tablets as corresponds to 800 μg. of digitoxin is weighed into a 15-ml. centrifuge tube and 10·00 ml. of methylene chloride is added. The tube is immediately closed with a cork covered with tinfoil and shaken in a machine for 30 minutes. Then the mixture is centrifuged at 2,000 r.p.m. for some minutes. 5·00 ml. of the clear solution is transferred to a beaker and the methylene chloride evaporated in a vacuum at 50°C. The residue containing the glycoside and any lubricant that may be present (stearic acid), is treated with 5 ml. of 50 per cent. ethyl alcohol and carefully warmed to about 70°C. for a few seconds. This warming is necessary to dissolve the glycosides under the prescribed conditions. The solution is cooled and the separated stearic acid removed by filtration. The filtrate is adjusted to 6·00 ml. with 50 per cent. ethyl alcohol. To this solution 2·00 ml. of 10 N sodium hydroxide solution is added. At the same moment as the alkali is added a stop watch is started and the extinction is determined at about 620 mμ every 30 seconds. The logarithms of the extinctions were plotted against time and a straight line is obtained. Log extinction at zero time is read from the graph. A standard curve is prepared from " zero " extinctions, obtained with weighed amounts (200 to 600 μg.) of digitoxin.

In this modification the Raymond method is unaffected by reasonable variations in alcoholic strength of the final solution and by temperature variations within the range 17° to 23°C. From measurements made on digitoxin, cymarin, strophanthin, ouabain, gitoxin, digitoxigenin, strophanthidin, and on the synthetic analogue β-phenyl-$\Delta^{\alpha:\beta}$-butenolide the extrapolated molar " zero " extinction coefficient has been found to be about 12,000 or $\log \varepsilon = 4{\cdot}08$ at 620 mμ. Lactose interferes, therefore it is preferable to extract digitoxin with methylene chloride, instead of with chloroform, from tablets containing this sugar. Injections, even those containing glycerol, are diluted with ethyl alcohol and directly estimated.

The Raymond method has the following advantages over the Knudson-Dresbach method. The absorption maximum lies far in the

red part of spectrum at 620 mμ, and thus yellow and red colours from impurities have a less serious effect on the measurements than in the Knudson-Dresbach method. The reagent is colourless and stable for some days. The measurements are made very rapidly. Another positive character is that the glycosides from *Scilla* and *Helleborus* do not give colours. A disadvantage is that it is necessary to work with speed in order to obtain sufficient points to construct the curve. The Raymond method, like the Knudson-Dresbach method, makes no distinction between glycosides and aglucones.

The Raymond method has been discussed more in detail than the other methods because in my opinion this method is the most promising of those acting on the lactone side chain. In combination with one of the methods discussed below acting on the sugar moiety, it seems possible to devise a good chemical assay of digitalis preparations.

A new reagent for digitalis glycosides has recently been proposed by Kedde[5]. He uses 3:5-dinitrobenzoic acid in alkaline solution to estimate the sum of glycosides and aglucones. The mechanism of the reaction is probably the same as in the Raymond reaction.

Another new reagent is sodium β-naphthoquinone-4-sulphonate used by Warren, Howland and Green[21] for the estimation of digitoxin. Ehrlich and Herter[22] and Feigl and Frehden[23,24] described the use of the reagent for the detection of active methylene groups. The colour produced is probably due to the formation of a quinoid condensation product. The condensation reaction is carried out in alkaline solution and the colour produced is purple. On acidification with acetic acid a stable yellow colour is obtained which may be extracted by dibutyl phthalate.

Warren, Howland and Green[21] assay digitoxin tablets in the following way. Tablets are ground to a fine powder, which is extracted with boiling chloroform. After filtering, the volume is adjusted and an aliquot part, corresponding to 200 μg. of digitoxin, is evaporated in an Erlenmeyer flask on a steam bath. 0·1 ml. of chloroform is added and the flask is shaken to wet all the residue. Then 4 ml. of alcohol and 0·5 ml. of 0·05 N sodium hydroxide solution are added. The flask is placed in a 100°C. bath and after 1 minute 1 ml. of the reagent (containing 0·024 per cent. of sodium β-naphthoquinone-4-sulphonate and 0·024 per cent. of sodium sulphite in distilled water) is rapidly added. One and one-half minutes after the addition of the reagent 0·5 ml. of acetic acid solution (containing 13 per cent. of acetic acid in alcohol) is added with agitation. The flask is removed from the bath, and cooled under the tap. The volume is adjusted to 25 ml. with alcohol and the transmission measured within 2 hours at about 450 mμ. A blank is prepared in the same manner omitting the digitoxin. As aldehydes give a positive reaction with the reagent, aldehyde-free ethyl alcohol must be used.

As expected, the authors report that lanatoside A (digitoxin derivative) and lanatoside C (digoxin derivative) gave a strong positive reaction. Curiously, however, gitoxin and its derivative lanatoside B gave only a weakly positive test. The only structural difference between digitoxin

and gitoxin is the additional hydroxyl group on C_{16}, and it is hard to believe that this hydroxyl group could interfere. The slight solubility of gitoxin may be responsible for the different behaviour of the glycoside. Digitonin gave a negative reaction.

Methods Dependent on the Sugar Moiety of the Glycosides

Many methods for the detection and determination of the sugars in the heart-active glycosides have been reported. All the digitalis glycosides of interest are built up according to the same scheme:—aglucone + 3 digitoxose (+ glucose). The strophanthus glycosides contain other sugars instead of digitoxose, for instance, ouabain contains rhamnose, strophanthin-k β-cymarose and glucose, and cymarin, cymarose.

On the basis of a sugar determination it is for the moment a technically easier task to devise an assay method which is specific for the glycosides and excludes the aglucones, than to construct one on the basis of a determination of the butenolide side chain. In the first case it is only necessary to extract the glycosides and the aglucones from an aqueous or ethyl alcoholic extract and to determine the sugars which are set free in a separate hydrolytic reaction. In the latter case it is necessary to separate glycosides and aglucones by a tedious process and then to estimate the glycosides. When working with a sugar method it is, however, always a good thing to remember that the part of the molecule actually measured is not the specific one. On principle those methods which depend on a reaction with the butenolide side chain should therefore be preferred.

Of the proposed colorimetric sugar methods only two recently described modifications of the Keller-Kiliani test will be discussed. The Keller-Kiliani test depends on the colours obtained when concentrated sulphuric acid is added to digitoxose dissolved in glacial acetic acid containing traces of ferric chloride. As early as 1906 Cloetta and Fischer[25] tried this method for the estimation of heart-active glycosides.

Recently James, Laquer and McIntyre[26] have described a modification that has been introduced in the U.S.P. XIII as Method II, Digitoxin Colorimetric Controls. They assayed digitoxin tablets in the following way. Ground tablets corresponding to 4 mg. of digitoxin were extracted overnight with 50 ml. of chloroform. The supernatant chloroform was filtered and the residue extracted 6 times with 5-ml. quantities of chloroform. The volume was adjusted and an aliquot part of the chloroform solution corresponding to 200 μg. of digitoxin was evaporated to dryness. To the residue were added 3 ml. of glacial acetic acid, 0·10 ml. of 5 per cent. ferric chloride solution ($FeCl_3.6H_2O$) and 0·25 ml. of concentrated sulphuric acid. The transmission was measured at 15-minute intervals until a maximum reading was reached, usually within 15 to 45 minutes. The light filter had a maximum transmission between 500 and 570 mμ. The Lambert-Beer law is fulfilled.

In assaying injections containing glycerol it is necessary to prepare a

special graph from measurements made on solutions containing known amounts of glycerol and digitoxin.

In a comprehensive study Soos[27] has investigated the details of the Keller-Kiliani test when applied to digitalis leaf. He used the reagent recommended by Lindewald[28,29], which consists of 97 ml. of glacial acetic acid, 2 ml. of concentrated sulphuric acid and 1 ml. of a 5 per cent. ferric chloride aqueous solution. Soos assays digitalis leaf in the following way. 1·50 g. of powdered leaf is moistened with a small quantity of water in a mortar and allowed to swell during 15 minutes. Then the mixture is washed into a flask with water and the weight adjusted to 151·5 g. The mixture is shaken now and then during 1 hour. After that 15 ml. of a 15 per cent. lead acetate solution is added. The precipitate is allowed to settle and the clear supernatant solution is filtered. 110 g. of the filtered solution, corresponding to 1·00 g. of the drug, is extracted with 3 × 25 ml. of chloroform. The chloroform solution is dried with sodium sulphate and filtered into an Erlenmeyer flask. The chloroform is distilled off and the residue dissolved in 10 ml. of freshly prepared reagent. The extinction is measured at 30-minute intervals and the maximum value recorded during 5 hours. As the reaction is sensitive to light it is preferable to let the solution stand in the dark during this time. The absorption curve has a maximum at 570 mμ.

Soos points out that unknown impurities in the glacial acetic acid he used had a very pronounced effect on the development of the colour. Thus it is necessary to construct a graph with the glacial acetic acid actually used. As standard substances either digitoxin or digitoxose may be used. Drawbacks of the methods are that the development of the colour takes 2 to 5 hours and that the extraction of the glycosides from the aqueous extract with chloroform is never quantitative. Usually about 80 per cent. is recovered. The presence of saponins in the drug decreases the yield obtained by extraction. Nevertheless, the method probably is the best of the known methods for assaying digitalis leaf. It is the only method that measures the glycosides present in the drug excluding the aglucones. But, as pointed out previously, the method has the defect of not measuring an essential part of the heart-active molecule.

The time seems to be near when the pharmacopœias will have seriously to discuss the problem of the assay of drugs containing heart-active glycosides in the light of the experience gained with chemical methods. A combination of a method assaying the total amount of glycosides and aglucones present, and a method assaying only glycosides would appear to define most of the necessary characteristics of the drugs.

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RESEARCH PAPERS

SYNTHETIC ŒSTROGENS

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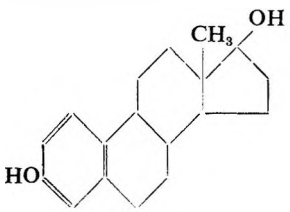
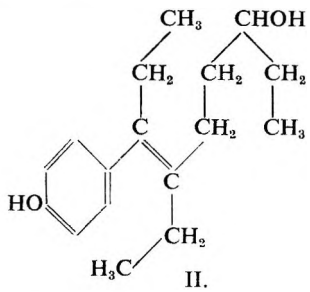
It is remarkable that all the most active synthetic œstrogens, with the exception of the œstrogenic carboxylic acids of the doisynolic acid type, possess two phenolic hydroxyl groupings, and in this respect differ from the potent natural œstrogen, œstradiol (I), which has one phenolic and one alcoholic hydroxyl. It was considered of interest, therefore, to synthesise the compound II in an attempt to obtain a synthetic œstrogen resembling, more closely, the œstradiol structure with regard to the chemical nature of the functional groupings.

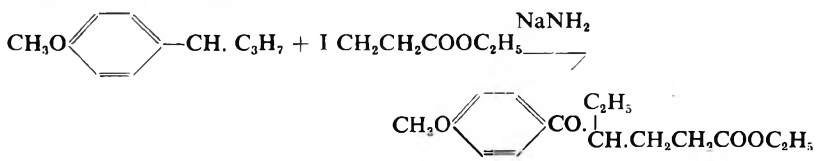


I.



II.

Attempts to synthesise 4-ethyl-5-(*p*-anisyl)-$\Delta^{4,5}$-heptenoic acid as an intermediate in the production of II were unsuccessful. Ethyl 4-(*p*-methoxybenzoyl)-caproate (III) was obtained from ethyl β-iodopropionate[1] and the sodium enolate of *p*-methoxybutyrophenone[2].



III.

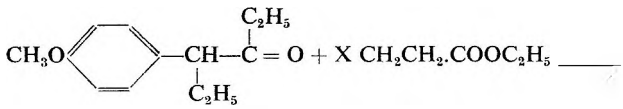
Treatment of III with one molar equivalent of ethyl magnesium iodide, or ethyl lithium, did not yield the anticipated hydroxyester. Similarly treatment of 4-(*p*-methoxybenzoyl)-caproic acid with three equivalents of ethyl magnesium iodide gave a theoretical recovery of the keto-acid.

An attempt to condense ethyl α(*p*-anisyl)-butyrate with diethyl succinate using sodium as the condensing agent yielded succinyl succinate as the sole product. Similar results were obtained in the attempted condensation of ethyl α-phenylbutyrate with succinic ester using alcohol-free sodium ethoxide and -phenylbutyryl chloride with succinic ester using triphenylmethyl sodium.

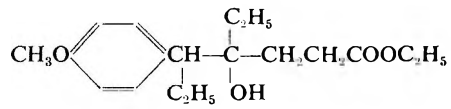
The successful reaction between 6-methoxytetralone and ethyl β-bromo-

propionate using magnesium in toluene to produce β-(6-methoxy-3:4-dihydronaphthyl-(1))-propionic acid[3], suggested that a similar reaction using 4-(*p*-anisyl)-3-hexanone (IV) in place of 6-methoxytetralone would yield the required intermediate.

The 4-(*p*-anisyl)-3-hexanone required was obtained by the action of diethyl cadmium on α-(*p*-anisyl)-butyryl chloride, the product being found to be identical with that formed by the action of ethyl iodide on *p*-methoxybenzyl-ethyl-ketone in the presence of alcohol-free sodium ethoxide[4]. On refluxing ethyl β-bromopropionate[5], magnesium and 4-(*p*-anisyl)-3-hexanone in toluene only a very poor yield of the product V was obtained. Substituting ethyl β-iodopropionate for the bromo analogue and conducting the experiment in ether gave a similar poor yield of V.



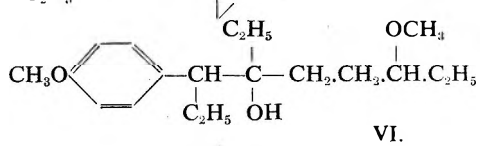
IV.



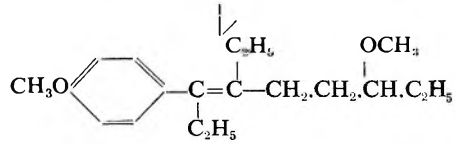
V.

An attempt to remove the elements of water from this by heating on a water-bath with 2 per cent. of iodine for 1 hour was unsuccessful.

The preparation of the Grignard complex from γ-methoxyamyl chloride[6] proved difficult, 6 hours' refluxing with activated magnesium in ether being required for completion of the reaction. Addition of 4-(*p*-anisyl)-3-hexenone to 2 equivalents of this Grignard complex gave, on working up in the normal way, a 67 per cent. yield of 3-(*p*-anisyl)-4-ethyl-7-methoxy-4-nonanol (VI). Dehydration by heating on a water-bath for 1 hour with 1 per cent. of iodine yielded an ethylenic compound. Ozonolysis of this compound gave an 88 per cent. yield of *p*-methoxypropiophenone, identified as its 2:4-dinitrophenylhydrazone and indicated VII as the structure of the olelene.



VI.



VII.

Demethylation of VII was achieved by heating with hydrogen bromide and acetic acid in an atmosphere of carbon dioxide for 2 hours. Purification of the product by adsorption chromatography on alumina yielded a viscous brown oil which contained no methoxyl and two active hydrogens. Carbon and hydrogen analysis agreed with that calculated for 3(*p*-hydroxyphenyl)-4-ethyl-7-hydroxy-$\Delta^{3,4}$-nonene (II). The failure to isolate the two possible geometrical isomers on chromatographic adsorption of II suggests that the product consisted of only one isomer. The use of iodine for the formation of the double bond and hydrogen bromide for demethylation of VII supplied conditions suitable for the interconversion of geometrical isomers and would be expected to produce the more stable form which in most cases of cis-trans pairs has the trans-configuration. It appears likely, therefore, that the compound II possesses the trans structure.

We are indebted to Professor Buttle and Dr. F. J. Dyer, of the Pharmacological Department of this School for the biological examination of compounds VII and II. The dimethyl ether VII was found to be inactive on administration in arachis oil solution of 20 mg. per 20 g. mouse. The phenolic alcohol II, in propylene glycol solution, was found to possess less than 1/40,000 of the activity of stilbœstrol.

Experimental

Ethyl 4-(p-*methoxybenzoyl*)*caproate.*

$$CH_3O.C_6H_4CO.\underset{\displaystyle C_2H_5}{\underset{|}{C}}HCH_2.CH_2COOC_2H_5.$$

The sodium enolate of *p*-methoxybutyrophenone was prepared by adding 50 g. of the ketone, dissolved in 50 ml. of dry benzene, in 10 aliquot parts at 10 minute intervals, to 12 g. of finely powdered sodamide in 250 ml. of dry benzene, the mixture being refluxed vigorously until no further ammonia was evolved. To the cooled benzene solution was added gradually, with rapid stirring, a solution of 55·6 g. of ethyl β-iodopropionate in 30 ml. of dry benzene, the reaction flask being kept cold in an ice bath. After completion of the addition, stirring was continued for 1 hour at room temperature and then with refluxing for 30 minutes. The cooled product was poured into 300 ml. of water, the benzene layer was separated and the aqueous layer extracted twice with benzene. The combined benzene solution and extracts were washed with sodium thiosulphate solution and finally dried over anhydrous sodium sulphate. Evaporation of the benzene and distillation of the residue *in vacuo* yielded a golden yellow oil, b.pt. 180° to 182°C./1·2 mm. Yield, 35 to 40 per cent. of theoretical. Found: Eq. Wt. 273; $C_{16}H_{22}O_4$ requires 278.

4-(p-*methoxybenzoyl*)*caproic acid.* $CH_3OC_6H_4CO.\underset{\displaystyle C_2H_5}{\underset{|}{C}}H.CH_2.CH_2.COOH.$

10 g. of the ethyl ester were refluxed with 100 ml. of N alcoholic potash for 3 hours. The cooled solution was diluted to 500 ml. with water and acidified with hydrochloric acid. The acid was filtered off and recrystallised from 40 per cent. aqueous alcohol, yielding 8·5 g. (94·5

per cent. of theory) of creamy white crystals, m.pt. 83·5° to 84°C. Found: C, 66·5; H, 6·86; CH_3O, 10·9 per cent., Eq. Wt. 250·8; $C_{14}H_{18}O_2$ requires C, 67·2; H, 7·2; CH_3O, 12·4 per cent., Eq. Wt. 250.

Diethyl p-*methoxyphenylethylmalonate.*

23·3 g. of sodium was added to 330 g. of absolute alcohol. After the solution had cooled to about 60° C. a mixture of 150 g. of ethyl oxalate and 194 g. of ethyl *p*-methoxyphenyl acetate[7] was added quickly with rapid stirring. The temperature, which dropped to about 40° C., was brought back to 60° C. and held at that temperature until the sodium salt of *p*-methoxyphenyl oxalacetic ester separated thickly. This occurred in about 2 or 3 minutes. The mixture was held at 60° C. for a further 30 minutes, after which the sodium salt was acidified with 70 g. of concentrated sulphuric acid diluted with 200 ml. of water. The mixture formed two layers. After verifying that the aqueous layer was acid to congo-red, the ester was taken into benzene, care being taken to avoid the crystallisation of sodium sulphate decahydrate by keeping the temperature above its melting-point. The benzene was distilled under reduced pressure. The distillation flask was then fitted with a still head carrying a thermometer dipping beneath the surface of the liquid, and an air condenser. The ester was heated on an oil bath, raising the temperature to 190° C. in 30 minutes and held at that temperature for a further hour. The crude *p*-methoxyphenylmalonic ester was then fractionated under reduced pressure yielding 235 g. (86·5 per cent. of theoretical) of a yellow oil. B.pt. 159° to 161°C./2 mm. Hg.

Diethyl p-*methoxyphenylethylmalonate.* $CH_3OC_6H_4.C(C_2H_5)(COOC_2H_5)_2$.

14·6 g. of sodium was dissolved in 175 ml. of absolute alcohol and to the solution of sodium ethoxide so formed was added, at 50° to 60° C., 180 g. of diethyl *p*-methoxyphenylmalonate. When the temperature had fallen to about 35° C., 103·75 g. of ethyl iodide were added as rapidly as possible and the temperature held at 35° C. for 6 hours with stirring. The alcohol was then removed by distillation, the residue diluted with 250 ml. of water and made acid to methyl red with acetic acid. A little sodium sulphite was added to remove any free iodine and the ester taken into benzene. After removal of the solvent the ester was distilled under reduced pressure, yielding 164 g. of a yellow oil with a boiling point identical with the starting material. Yield, 82·5 per cent. of theoretical. Eq. Wt. found 147·7; $C_{15}H_{22}O$ requires 147.

α-(p-*Anisyl*)-*butyric acid.* $CH_3OC_6H_4CH(C_2H_5)COOH$.

100 g. of *p*-methoxyphenylethylmalonic ester were saponified by refluxing on a water-bath with 750 ml. of 10 per cent. alcoholic potash for 3 hours. The cooled solution was diluted with 3 l. of water and acidified with hydrochloric acid and the precipitated acid filtered off at the pump. Evaporation of the filtrate to about half its volume gave, on cooling, a fourth crop of crystals. Recrystallisation from light petroleum (40° to 60°C.) yielded 63 g. of needle-shaped crystals, m.pt. 64° to 65°C. Yield, 92 per cent. of theoretical. Found: C, 68·5; H, 7·3; CH_3O, 14·9 per cent., Eq. Wt. 193. $C_{11}H_{14}O_3$ requires C, 68·8; H, 7·2; CH_3O, 16·0 per cent., Eq. Wt. 194.

α-(p-Anisyl)-butyryl chloride. $CH_3OC_6H_4CH(C_2H_5)COCl$.

39 g. of α-(*p*-anisyl)-butyric acid was refluxed with 70 g. of thionyl chloride for 3 hours. The excess of thionyl chloride was distilled off and the residue distilled under reduced pressure, yielding a pale yellow pungent liquid. B.pt. 120°C./4 mm. Hg. pressure. Yield 40 g., 94 per cent. of theoretical.

4-(p-Anisyl)-3-hexanone. $CH_3O.C_6H_4CH(C_2H_5).CO.C_2H_5$.

Ethyl magnesium bromide was prepared from 13·6 g. of ethyl bromide and 3 g. of magnesium in 100 ml. of absolute ether. The solution was cooled in an ice bath and 12 g. of dry, finely powdered cadmium chloride added. After refluxing for 45 minutes the mixture gave no colour with Michler's ketone indicating the absence of any Grignard complex. The reflux was replaced by a condenser set for distillation and nearly all the ether removed by heating on a water-bath. 50 ml. of dry benzene was then added and 20 ml. distilled. A further 50 ml. of dry benzene was then added to the mixture. The reflux condenser was replaced and to the cooled benzene solution of diethyl cadmium, was added, with stirring, a solution of 21·3 gm. of -(*p*-anisyl)-butyryl chloride in 20 ml. of benzene, during about 3 minutes. The mixture was stirred at room temperature for 15 minutes and then at 40°C. for 2 hours. The cooled reaction mixture was decomposed by pouring on to 150 g. of ice and 70 ml. of 2N sulphuric acid. The benzene layer was separated and the lower aqueous layer shaken with three further quantities of benzene. The combined benzene solution and extracts were washed with dilute sodium carbonate solution and finally dried over anhydrous sodium sulphate. The benzene was removed under reduced pressure and the crude ketone fractionated, yielding a colourless oil boiling at 132° to 134°C./2·5 mm. Hg. pressure. Yield, 71 per cent. of theoretical.

Semicarbazone: m.pt. 131·5° to 132°C. Found: C, 64; H, 8·05; N, 15·4 per cent.; $C_{14}H_{21}N_3O$ requires C, 63·8; H, 7·96; N, 15·9 per cent.

2:4-Dinitrophenylhydrazone: m.pt. 91·5 to 92°C. Found: C, 58·8; H, 5·7; N, 14·8 per cent.; $C_{19}H_{21}N_4O_5$ requires C, 59·25; H, 5·45; N, 14·5 per cent.

3-(p-Anisyl)-4-methyl-$\Delta^{3,4}$-hexene, $CH_3O.C_6H_4.C(C_2H_5):C(CH_3).C_2H_5$.

5·15 g. of 4-(*p*-anisyl)-3-hexanone, dissolved in 20 ml. of absolute ether, was added during 15 minutes, with cooling and stirring, to 1·5 equivalents of methyl magnesium iodide in 30 ml. of ether. The mixture was then refluxed for 3 hours, cooled, and then decomposed by pouring on to 50 g. of ice and 35 ml. of dilute sulphuric acid. The ethereal layer was separated and the aqueous portion extracted thoroughly with ether. The combined ethereal solution and extracts were washed with sodium thiosulphate solution and dried over anhydrous sodium sulphate. Distillation yielded a colourless oil boiling at 99° to 105°C./0·8 mm. Hg. pressure. Yield 3·38 g.

Distillation, under reduced pressure, of this oil with 2 per cent. of iodine and purification of the distillate in the normal manner yielded a colourless oil distilling at a bath temperature of 95° to 100° C. and 0·05

mm. Hg. pressure. Found C, 81·56; H, 9·3; CH_3O, 15·3 per cent.; $C_{14}H_{20}O$ requires C, 82·3; H, 9·8; CH_3O, 15·2 per cent.

The compound contained no active hydrogen.

Demethylation of 3-(p-*Anisyl)-4-methyl-$\Delta^{3,4}$-hexene.*

1·5 g. of the above methoxy compound was heated with three equivalents of methyl magnesium iodide under a reflux condenser, the temperature being raised slowly to 165°C. and then maintained at this temperature for 3 hours. The cooled reaction mixture was then decomposed with ice and dilute sulphuric acid, the ethereal layer separated and the aqueous layer extracted with ether. The combined ethereal solution and extracts were washed with sulphate solution and then extracted with dilute aqueous sodium hydroxide. Acidification of the alkaline extract with dilute sulphuric acid, extraction with ether and distillation yielded a yellow glass distilling at 108° to 118°C./0·8 mm. Hg. pressure.

Ethyl 4-ethyl-5-(p-*anisyl)-4-hydroxyheptanoate.*

$$CH_3OC_6H_4.\underset{|\atop H_5C_2}{CH}.\underset{|\atop C_2H_5}{C}(OH)(CH_2)_2COOC_2H_5.$$

A. 5·15 g. of 4-(*p*-anisyl)-3-hexanone was refluxed with 0·61 g. of magnesium and 4·43 g. of ethyl β-bromopropionate in 50 ml. of dry toluene. Addition of 0·3 g. of iodine started the reaction. After 3 hours refluxing, the mixture was cooled and decomposed with ice and dilute sulphuric acid, the toluene layer separated and the aqueous layer extracted with ether. The combined toluene solution and ethereal extracts were washed with dilute sodium thiosulphate solution and finally dried over anhydrous sodium sulphate. Distillation yielded 0·38 g. of a light yellow, semi-solid substance boiling at 145° to 155°C./0·3 mm. Hg. pressure.

B. As method A, except for the use of ethyl β-iodopropionate in place of the β-bromo analogue and ether instead of toluene. 6 hours refluxing was required for solution of the magnesium. On work-up, as before, there was obtained a yellow semi-solid fraction distilling at 115° to 120°C./0·05 mm. Hg., which proved to be the same as that obtained in A. Yield, 0·55 g. Found: C, 69·7; H, 8·7; CH_3O, 10·7 per cent.; $C_{18}H_{25}O_4$ requires C, 70·1; H, 9·08; CH_3O, 10·05 per cent.

3-(p-*Anisyl)-4-ethyl-7-methoxy-4-nonanol.*

$$CH_3OC_6H_4.CH.(C_2H_5).\underset{\cdot\atop C_2H_5}{C}(OH).(CH_2)_2.CH.(OCH_3)C_2H_5$$

1·2 g. of magnesium turnings, 30 ml. of dry ether and 6·8 g. of freshly distilled γ-methoxyamyl chloride were refluxed together, with stirring. Two or 3 drops of methyl iodide helped to initiate the reaction which was very slow, requiring 6 hours for completion. To the cooled ethereal solution of γ-methoxyamyl magnesium chloride was added, during 30 minutes, 5·15 g. of 4-(*p*-anisyl)-3-hexanone dissolved in 10 ml. of dry ether. Stirring was continued and the mixture refluxed for 4 hours. Decomposition of the complex with ice and dilute sulphuric acid, yielded,

on working up, 5·15 g. of a viscous yellow oil boiling at 140° to 145°C./0·04 mm. Hg. pressure. Found: C, 74·3; H, 10·38; CH_3O, 20·06 per cent.; $C_{19}H_{32}O_3$ requires C, 74·02; H, 10·39; CH_3O, 20·13 per cent.

3-(p-Anisyl)-4-ethyl-7-methoxy-$\Delta^{3,4}$-nonene.

$CH_3OC_6H_4.C(C_2H_5):C(C_2H_5).(CH_2)_2.CH(OCH_3).C_2H_5$

4·5 g. of 3-(*p*-anisyl)-4-ethyl-7-methoxy-4-nonenol and 50 mg. of iodine were heated together on a water-bath for 1 hour under a reflux air condenser. The resultant dark brown oil was dissolved in ether, washed with sodium thiosulphate solution and the ethereal solution then dried over anhydrous sodium sulphate. Distillation yielded a light yellow oil, boiling at 130° to 135°C./0·1 mm. Hg. pressure. Found: C, 78·08; H, 10·08; CH_3O, 21·48 per cent.; $C_{19}H_{30}O_2$ requires C, 76·63; H, 10·34; CH_3O, 21·38 per cent.

Ozonised oxygen was passed into a solution of 0·5 g. of the above ethylenic compound in 50 ml. of chloroform for the calculated time. The chloroform was then removed under reduced pressure and the ozonide decomposed with iced water. Extraction with ether and evaporation of the solvent yielded a semi-solid residue. This was dissolved in light petroleum (40° to 50°C.) and passed through a column of activated alumina. The chromatogram showed three zones, (1) a narrow dark band at the top, (2) a broad pale-yellow zone, (3) a narrow band exhibiting a blue fluorescence under ultra-violet light, near the bottom. Washing with light petroleum removed the lowest band, but continued washing did not appear to remove the yellow zone. Elutriation with benzene and ether removed the yellow zone leaving a small dark brown band at the surface. Evaporation of the solvents yielded:

(a) 0·14 g. of a white wax-like substance which was insoluble in methyl and ethyl alcohols and water.

(b) 20 mg. of an oil which gave a red oil on addition of a saturated alcoholic solution of 2:4-dinitrophenylhydrazone sulphate. Crystallisation of this oil could not be brought about.

(c) 0·25 g. of an oil which yielded a 2:4-dinitrophenylhydrazone melting at 185° to 187°C.

Mixed melting-point with a sample of *p*-methoxypropiophenone 2:4-dinitrophenylhydrazone, 186° to 187° C.

3-(p-Hydroxy)-4-ethyl-7-hydroxy-$\Delta^{3,4}$-nonene.

$HOC_6H_4.C(C_2H_5):C(C_2H_5).(CH_2)_2CH.(OH).C_2H_5$.

0·5 g. of 3-(*p*-anisyl)-4-ethyl-7-methoxy-$\Delta^{3,4}$ nonene was heated in an oil bath at 140° C. for 2 hours, with 1·5 ml. of 47 per cent. hydrobromic acid and 6 ml. of glacial acetic acid in an atmosphere of carbon dioxide. The reaction mixture was then cooled, diluted with water and made just alkaline with sodium hydroxide solution, keeping the mixture cold in an ice bath. The brown semi-solid product was taken into ether, the ethereal solution washed with water and finally dried. On distillation, 0·4 g. of a viscous brown oil was obtained.

A solution of this in 40 ml. of cyclohexane was passed through a column of activated alumina. Development of the chromatogram with

benzene revealed a narrow dark brown band at the top of the column, below this a broad yellow zone and at the bottom a narrow band exhibiting a blue fluorescence under ultra-violet light. Continued elutriation with benzene removed the blue-fluorescent band. A mixture of equal parts of benzene and acetone removed the yellow zone.

Distillation of this latter elutriate yielded 270 mg. of a very viscous oil which could not be crystallised. Found: C, 77·94; H, 9·91; CH_3O, 0 per cent.; $C_{17}H_{16}O_2$ requires C, 77·86; H, 9·92; CH_3O, 0 per cent.

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THE PHARMACOLOGICAL ACTIONS OF *ERYTHRÆA SPICATA*

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ERYTHRÆA SPICATA is an annual herb belonging to the family Gentianaceæ. It grows wild in damp places specially in rice fields. Muschler[1] described this plant and other species growing in Egypt, where the public has used extracts as an antidote for scorpion sting, but one of us[2] has shown that it has no practical effect for this purpose. Gaston Bonnier[3] (1886) examined the European species *Erythræa Centaurium*. He found that it contains two glycosides and stated that it was antipyretic. Washburn[4] stated that it contained a bitter principle similar to that in gentian and stated that it was a diaphoretic and febrifuge. Gathercoal[5] said that it contained a glycoside, erythrocentaurin, and a resin.

Recently, attention was drawn to *Erythræa spicata* as it is believed to be efficacious in hypertension. The present work was therefore undertaken.

Experimental

Preparation of solutions.—An alcoholic extract of the plant was concentrated to a semi-solid consistency so that 0·3 g. of concentrated extract corresponded to 1 g. of the powdered plant. This concentrated extract (alcohol free) was dissolved in Ringer's or Locke's solution to the required concentration and the *p*H of the solution was adjusted before the experiment to *p*H 7·6.

Toad's Heart.—The method used was a modification of Syme's method of perfusion. The drug produced depression in the force of

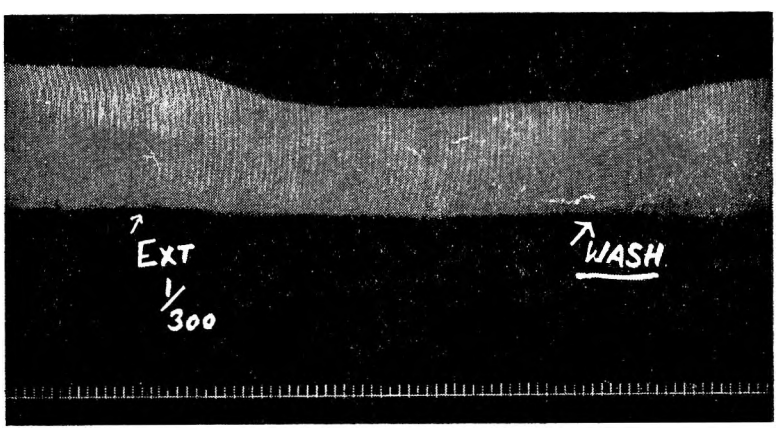

Fig. 1. Effect of extract of *Erythræa spicata* extract (1 in 300) on toad's heart.

contraction of the heart (Fig. 1). The lowest concentration which just produced depression of the heart was 1 in 600 of the extract. The same effect was produced by the drug after atropinising the heart.

Rabbit's Heart.—A modification of Gunn's method was used. No change in the amplitude or rate of the heart was demonstrated by 1 in 1000 but there was an increase in the coronary outflow varying from 30 to 40 per cent.

Blood Vessels.—Trendelenberg's method was used for perfusion of toad's vessels. The drug produced definite dilatation of blood vessels in dilutions between 1 in 100 and 1 in 5000 of the extracts. Solutions of 1 in 100 produced an increase of the flow varying from 68 per cent. to 100 per cent. averaging 86 per cent. and 1 in 1000 produced an increase varying from 39 per cent. to 73 per cent. with an average of 58 per cent. and 1 in 5000 produced an average increase of 19 per cent.

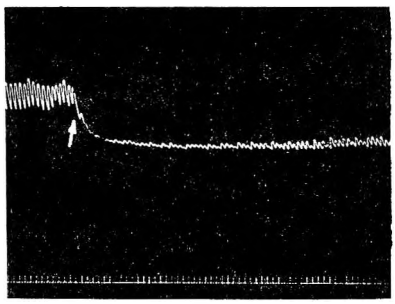

FIG. 2. Effect of *Erythræa spicata* extract (1 in 4500) on rabbit's intestine.

Intestine.—A modified Magnus technique was employed. The drug produced definite relaxation of the intestine of the rabbit (Fig. 2). Dilutions of 1 in 1000 produced in some cases very strong inhibition of the contractions, while 1 in 10,000 produced just noticeable depression.

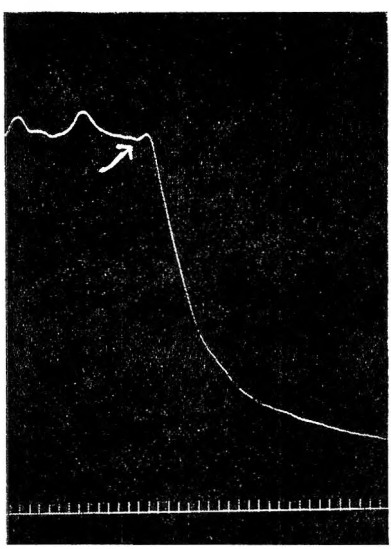

FIG. 3. Effect of *Erythræa spicata* extract (1 in 1000) on guinea-pig's uterus.

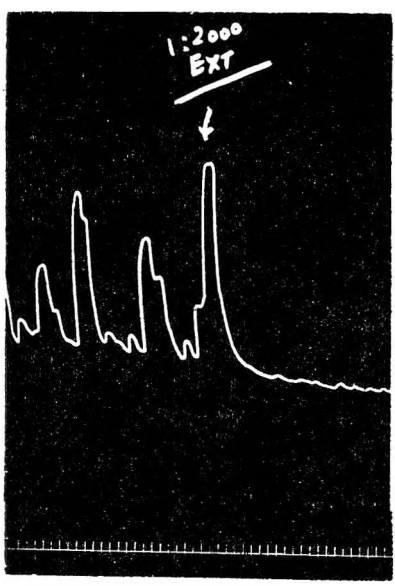

FIG. 4. Effect of *Erythræa spicata* extract (1 in 2000) on rabbit's uterus.

Uterus.—The drug produced definite relaxation of the rabbit's and guinea-pig's uteri whether pregnant or not, in all dilutions tested which ranged from 1 in 8000 to 1 in 1000 (Figures 3 and 4).

Action on Blood Pressure.—Dogs anæsthetised by chloralose intravenously after preliminary ether anæsthesia were used. The blood pressure was recorded from the carotid artery by mercury manometer on smoked paper in the ordinary way. The drug was injected intravenously through a cannula into the jugular vein. The drug produced a definite fall of blood pressure; the greater the dose injected, the greater the fall of blood pressure and the longer the duration of the fall. Experimental results in two animals may be quoted. (a) In a dog of 15 kg. body weight injected with 0·3 g. of extract, the blood pressure reading was reduced from 185 mm. Hg to 155; 1·5 g. reduced it to 115 mm. Hg and 3 g. to 95 mm. Hg.

(b) In a dog of 7 kg. body weight 3 g. of extract reduced the blood pressure reading from 155 to 65 mm. Hg. The reading returned to 100 mm. in 10 minutes then to 138 mm. in 70 minutes and to 142 mm. in 100 minutes.

The fall of blood pressure produced by the drug was not affected by atropine, indicating that the fall was not due to stimulation of the cholinergic nerve endings. After each injection of the drug there was acceleration of the heart beats and of the respiration, probably as a result of the fall in blood pressure (Fig. 5).

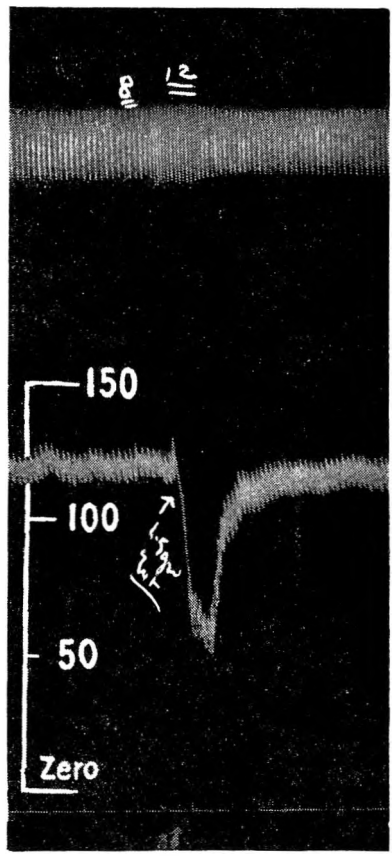

FIG. 5. Dog 7·5 kg. Effect of 1·5 g. of extract on respiration (upper tracing) and blood pressure (lower tracing).

Toxicity.—Groups of experimental animals i.e. toads, rats and dogs were injected with relatively large doses of the extract without any toxic effects. The following doses were injected without toxic manifestation. (1) Up to 1·2 g. of extract per 100 g. in the ventral lymph sac of toad. (2) Up to 2 g. of extract intramuscularly per kg. of rat. (3) Up to 0·6 g. of extract intravenously per kg. of dog.

Moreover, intramuscular injections of the extract in rabbits in doses of 0·25 g./kg. every day for 15 days produced no apparent toxic manifestations and no pathological changes in the internal organs on killing the animals.

The Active Principle

An attempt to isolate the active principle by purifying the extract with lead acetate, and precipitating it from alcoholic solution by ether resulted in an amorphous hygroscopic light brownish powder. This powder was proved to be a glycoside, readily soluble in water or dilute alcohol but insoluble in ether, chloroform, ethyl acetate and absolute alcohol. It had the pharmacological activities of the extract and it formed about 1·6 per cent. of the powdered plant. It did not lose its effects after treatment with hydrochloric acid.

Discussion

Erythræa spicata produces some depression of the force of contraction of the heart in dilutions of 1 in 600 to 1 in 100 of extract. This was not due to vagus stimulation as it occurred after administration of atropine. Dilutions weaker than 1 in 600 of the extract produce practically no depression. In rabbits it produces a definite increase in the coronary flow. It produces definite relaxation of the plain muscle in general. This was shown in: (a) The dilatation of the blood vessels of the toad when perfused with solutions of the drug. The percentage increase in the flow varies from an average of 86 per cent. which was produced by 1 in 100 of the extract, to 19 per cent. which was produced by 1 in 5000 concentration. A moderately good increase in the flow of average 58 per cent. was produced by 1 in 1000. This last concentration did not depress the perfused heart. (b) The powerful relaxation of the rabbits' intestine, in dilutions of 1 in 1000, diminishing both tone and contractions. (c) Definite relaxation of the rabbits' and guinea-pigs' uteri whether pregnant or not.

The drug produces a definite fall of blood pressure; 0·2 g. of the extract per kg. of body weight given intravenously produces an average fall of blood pressure of 46 per cent. of the normal, and 0·04 g. of the extract per kg. of body weight produced an average fall of blood pressure 19 per cent. of the normal. This last dose will make a concentration in the blood of nearly 1 in 2000. This concentration did not depress the perfused heart, so the fall in blood pressure is presumably due to the effect on the blood vessels. The drug is not toxic even if given in relatively large doses. Hydrolysis did not destroy the activity of the drug.

The experiments recorded show that this drug may be of value for reducing blood pressure. At the same time it dilates the coronaries and has a low toxicity, while the effect on the heart in the usual doses is slight. It seems to be worthy of clinical trial as an anti-spasmodic and in cases of hypertension, possibly also in angina pectoris.

Summary

1. The drug produced depression of the toad's heart at a concentration of 1 in 600 of the extract; below this concentration there was practically no depression.

2. It produced increase in coronary flow in the rabbit's heart.

3. It produced fall of blood pressure in dogs and dilatation of vessels in toads.

4. It depressed plain muscle.

5. The active principle is glycosidal in nature.

6. It has a very low toxicity.

Our thanks are due to Professor I. Shawki Bey for suggesting the problem, and to Dr. M. K. Madkour for helping in some of the experiments.

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THE DETERMINATION OF AMINO-COMPOUNDS OCCURRING AS IMPURITIES IN PHARMACEUTICAL CHEMICALS

PART II.

3-Amino-4-Hydroxyphenylarsonic Acid in Acetarsol

By C. W. Ballard

From the Analytical Control Division of May and Baker, Ltd.

Received November 30, 1948

In connection with the manufacture of acetarsol, a quantitative method was required for the determination of traces of 3-amino-4-hydroxyphenylarsonic acid and it was thought that a suitable method might be based on the official limit test for this impurity. This depends upon diazotisation and coupling as does the test for arsanilic acid in tryparsamide and hence, in view of the sources of error revealed in the latter[1] and in the quantitative method[2] proposed by MacDonald and Reynolds, it was decided to make a thorough study of the conditions and reactions involved.

The Official Test Colour Standard

The colour standard of the official test is not obtained directly from 3-amino-4-hydroxyphenylarsonic acid but from the product of hydrolysis of acetarsol with hydrochloric acid. Bonino[3], who describes a colour test for acetarsol involving heating with hydrochloric acid, suggests that an arsenoso-compound is first formed, whilst Phillips[4] found that acetarsol yielded 4-bromo-2-aminophenol when heated with hydrobromic

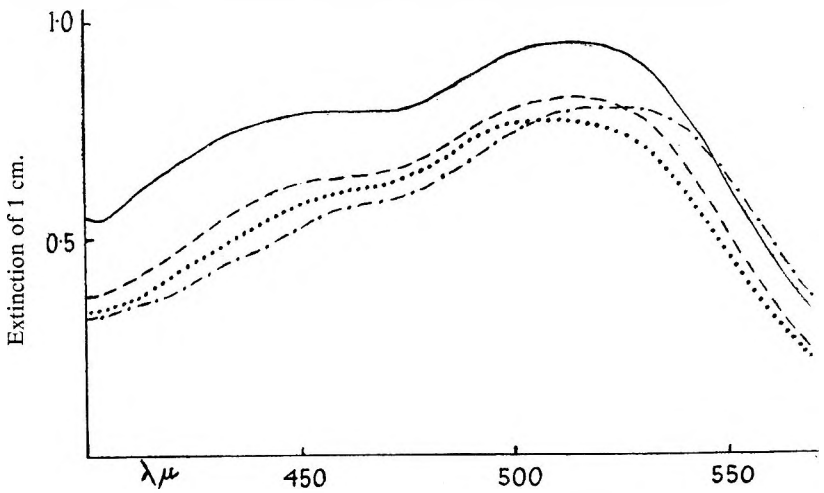

Fig. 1. Spectrophotometric absorption curves of β-naphthol azo-dyes
——— 3-amino-4-hydroxyphenylarsonic acid (0·4 mg. = 1·7 micromol)
— — — hydrolysis product of acetarsol (0·4 mg. = 1·45 micromol)
····· 2-aminophenol (1·7 micromol)
·— ·— 4-chloro-2-aminophenol (1·7 micromol)

acid. Hence it appeared desirable to confirm that hydrolysis of acetarsol under the conditions specified in the official test does yield 3-amino-4-hydroxyphenylarsonic acid.

If the arsonic acid group were removed arsenate would be present, but none was detected by a test, using magnesia mixture, of known high sensitivity; more conclusive evidence was obtained from the spectrophotometric absorption curves of the azo-dyes obtained by diazotising and coupling with β-naphthol. From Figure 1 it is seen that the curve for the product of hydrolysis of acetarsol closely follows that for 3-amino-4-hydroxyphenylarsonic acid and the ratio of the colour intensities is approximately that of the molecular weights (mol. wt. of acetarsol/ mol. wt. of 3-amino-4-hydroxyphenylarsonic acid = 1/0·85). On the other hand the curves for 2-aminophenol and 4-chloro-2-aminophenol are somewhat different in shape and moreover the colour intensities are appreciably less than those for an equivalent weight of 3-amino-4-hydroxyphenylarsonic acid. Hence it was concluded that the latter is in fact the product of hydrolysis of acetarsol; however, since it was readily available and a simple method of purifying it by recrystallisation is described by Ehrlich and Bertheim[5], it was used in subsequent work because more convenient.

Optimum Conditions for Diazotisation and Coupling of 3-amino-4-hydroxyphenylarsonic Acid

Optimum conditions for diazotisation and coupling were determined in the usual way, an interesting feature of the results being the effect of *p*H on the rate of coupling and on the colour obtained. With decrease in alkalinity coupling became slower and the violet component of the colour increased gradually but at the point at which β-naphthol began to precipitate a sharp change to a definite violet occurred. These colour changes may be associated with the presence of a hydroxyl group in the dye molecule.

The following method embodies the optimum conditions, and a calibration curve obtained by this method is shown in Figure 3. To 5 ml. of solution containing not more than 0·25 mg. of 3-amino-4-hydroxyphenylarsonic acid add 5 ml. of N/1 hydrochloric acid, cool to about 5°C., add 2 ml. of freshly prepared 0·1 per cent. solution of sodium nitrite and mix. After 2 minutes add 0·05 g. of sulphamic acid, shake well and leave in the ice-bath 5 minutes. Add 10 ml. of previously cooled β-naphthol solution (freshly prepared 5 per cent. solution of recrystallised β-naphthol in 2N sodium hydroxide) and mix. Leave in the ice-bath for 10 minutes, and then place in water at 20°C. for 5 minutes. Read the extinction of 2 cm. using an Ilford 604 filter. Subtract the value of the blank reading obtained in a test omitting the 3-amino-4-hydroxyphenylarsonic acid.

The Official Test—Coprecipitation

Since in the case of tryparsamide low results were obtained by precipitating the tryparsamide and applying the test for arsanilic acid to

the filtrate, it was expected that a similar effect would result with acetarsol. To determine the extent of the loss by coprecipitation in the official test, it was modified slightly as follows to adapt it for photoelectric absorptiometry. Furthermore, sodium carbonate was substituted for sodium hydroxide to avoid hydrolysis of the acetarsol since, in the case of chloracetylaminophenylarsonic acid, appreciable hydrolysis to arsanilic acid had been found to occur in sodium hydroxide solution. Dissolve 0·2 g. in a mixture of 1 ml. of N/1 sodium carbonate and 8 ml. of water. Add 1 ml. of 5N hydrochloric acid, mix, filter, using reduced pressure, to dryness, wash the precipitate with 1 ml. of water and use 1 ml. of water to wash the filter. Cool the mixed filtrate and washings, diazotise and couple with β-naphthol and read the extinction as already described.

This test was applied to a sample of acetarsol which contained about 0·06 per cent. of 3-amino-4-hydroxyphenylarsonic acid; the acetarsol filtered off was then again submitted to the test and this procedure repeated several times. The extinction values (corrected for reagent blank) obtained are shown in Figure 2 and it is seen that the extinction values tend to a constant value of about 0·075, which is shown later to be not entirely due to coprecipitation.

Consideration was then given to devising a test avoiding filtration, especially as it seemed unlikely that the acetarsol would enter into any chemical reactions during the test. Using the same material as used above, optimum conditions were determined and the following provisional test decided upon. Dissolve 0·2 g. in a mixture of 1 ml. of N/1 sodium carbonate and 8 ml. of water and add 1 ml. of 5N hydrochloric acid. Cool to about 5°C., add 2 ml. of freshly prepared 0·5 per cent. solution of sodium nitrate and mix. Complete the test as already described.

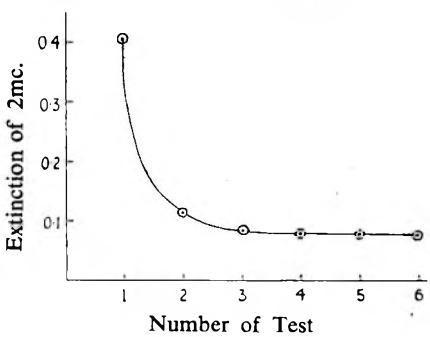

FIG. 2. Results obtained in successive tests by precipitation and filtration on the same material

Using this method an extinction of 0·44 was obtained, compared with 0·405 for the first value in the precipitation tests (Fig. 2). However, to put the test on a firm basis it was desirable to prepare a sample of pure acetarsol; in addition the constant extinction value of 0·075 reached in the precipitation series required explanation.

PURIFICATION OF ACETARSOL AND RECOVERY OF ADDED 3-AMINO-4-HYDROXYPHENYLARSONIC ACID

From Figure 2 it is seen that precipitation by acid from an alkaline solution is a relatively effective method of purifying acetarsol. Material from the same sample as used above was repeatedly submitted to this treatment, 7 precipitations being made at room temperature from sodium

carbonate solution by adding an exactly equivalent amount of diluted hydrochloric acid, followed by 4 precipitations from ice-cold sodium bicarbonate solution with ice-cold diluted hydrochloric acid. The last 2 specimens were tested by the method described above but using varying conditions for effecting solution and precipitation; identical results were obtained on both specimens and are shown in Table I.

TABLE I

Conditions used to dissolve and precipitate acetarsol		Extinction Values
Solution	Precipitation	
a 0·1 g. of sodium bicarbonate in 5 ml. of water at 5°C.	5 ml. of N/1 hydrochloric acid at 5° C.	0·025
b As above but at room temperature	As above but at room temperature	0·04
c 1 ml. of N/1 sodium carbonate and 4 ml. of water at room temperature	As above	0·055
d 1 ml. of N/1 sodium hydroxide and 4 ml. of water at room temperature	As above	0·07

It was concluded that these two specimens of acetarsol were as pure as could be obtained by the reprecipitation method. Furthermore, it was clear that hydrolysis of acetarsol to 3-amino-4-hydroxyphenylarsonic acid occurs even under relatively mild alkaline conditions and probably also in acid solution. That hydrolysis occurs also in boiling water was shown by recrystallising the purified material from boiling water when the excellent crystals obtained were found to contain about twice as much amino-compound as the original material.

It was now possible to explain the constant value obtained in Figure 2. The extinction value of 0·075 would be made up of about 0·05 from amino-compound produced by hydrolysis during the test itself (Table I, c), and about 0·025 from amino-compound already present in the acetarsol, having been derived from acetarsol by hydrolysis under the conditions of solution and precipitation, and coprecipitated with it.

Calibration curves for 3-amino-4-hydroxyphenylarsonic acid (a) alone, prepared by the method already described, and (b) in the presence of acetarsol, prepared by the proposed method described later, are shown in Figure 3 and it is seen, as expected, that low recovery is obtained in the presence of acetarsol owing to coprecipitation. It is, therefore, necessary to use either internal standards or a calibration curve prepared with acetarsol; for the latter it is sufficient to use a specimen of only relatively low amino-compound content.

Proposed Methods

(a) Photoelectric absorptiometer. Prepare a calibration curve by the method described below using, instead of 5 ml. of water, 5 ml. of solution containing up to 0·25 mg. of 3-amino-4-hydroxyphenylarsonic acid; from each value subtract that obtained on the acetarsol alone. The determination on a sample is as follows. To 5 ml. of water at about 5°C. add 0·1 g. of sodium bicarbonate and 0·2 g. of acetarsol. Shake to dissolve and add 5 ml. of N/1 hydrochloric acid previously cooled to about 5°C. Add

2 ml. of freshly prepared 0·5 per cent. solution of sodium nitrite and mix. After 3 minutes add 0·05 g. of sulphamic acid, shake well and leave in the ice-bath for 5 minutes. Add 10 ml. of previously cooled β-naphthol solution (freshly prepared 5 per cent. solution of recrystallised β-naphthol in 2N sodium hydroxide) and mix. Leave in the ice-bath for 10 minutes and then place in water at 20°C. for 5 minutes. Read the extinction of 2 cm. using Ilford 604 filter and subtract the value of a reagent blank together with 0·025. Read the amount of 3-amino-4-hydroxyphenylarsonic acid from the calibration curve.

(b) Tintometer. Prepare a calibration curve relating red units and

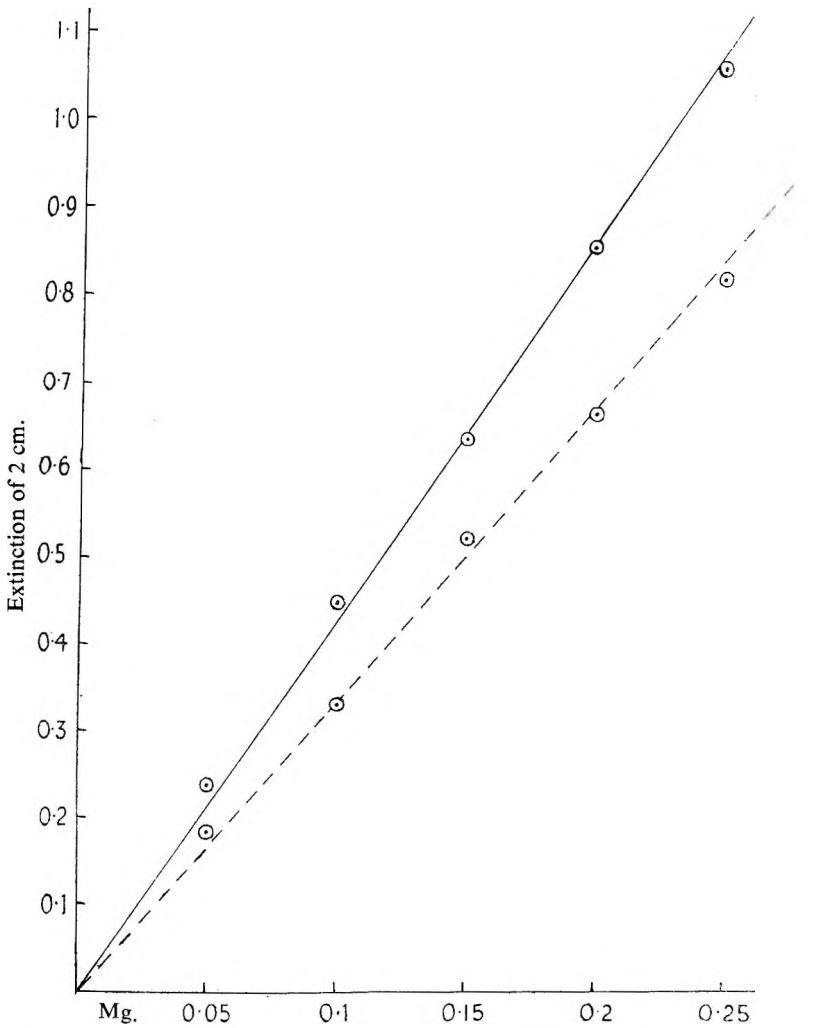

FIG. 3. Calibration curves for 3-amino-4-hydroxyphenylarsonic acid
——— alone
— — — in the presence of acetarsol

3-amino-4-hydroxyphenylarsonic acid by the method described under (a) above (using a 1 cm. cell 0·25 mg. gives about 5 red units). In testing samples, proceed as under *(a)* but subtract 0·1 red unit from the 1 cm. cell reading before reading off from the calibration curve.

(c) British Pharmacopœia Limit Test. The calculated official limit is 0·085 per cent. but, owing largely to the coprecipitation occurring, the actual limit is about 0·13 per cent. Thus the sample used in much of the above work contained by the proposed method 0·06 per cent., whereas by a method closely following the official test as regards preparation of solution for testing only about 0·04 per cent. was found. From Figure 3 it can be calculated that a sample of acetarsol containing 0·13 per cent. of 3-amino-4-hydroxyphenylarsonic acid would give, by the proposed test, a colour equal to that produced by 0·21 mg. of 3-amino-4-hydroxyphenylarsonic acid and hence a simple revised limit test with the same effective limit may be applied as follows. To 5 ml. of water at about 5°C. add 0·1 g. of sodium bicarbonate and 0·2 g. of acetarsol. Shake to dissolve and add 5 ml. of N/1 hydrochloric acid previously cooled to about 5°C. Add 2 ml. of freshly prepared 0·5 per cent. solution of sodium nitrite and mix. After 3 minutes add 0·05 g. of sulphamic acid, shake well and leave in the ice-bath 5 minutes. Add 10 ml. of previously cooled β-naphthol solution and mix. Leave in the ice-bath for 10 minutes and then place in water at 20°C. for 5 minutes. The red colour developed is not greater than that produced in the following way. To 5 ml. of a solution containing 0·21 mg. of 3-amino-4-hydroxyphenylarsonic acid add 5 ml. of N/1 hydrochloric acid and cool to about 5°C. Add 2 ml. of freshly prepared 0·1 per cent. sodium nitrite solution and complete the test as described above.

A solution of 3-amino-4-hydroxyphenylarsonic acid in N/2 hydrochloric acid containing 0·21 mg. in 10 ml. may be prepared by dissolving 0·0124 g. of acetarsol in a mixture of 21 ml. of hydrochloric acid and 21 ml. of water and boiling under a reflux condenser for 5 minutes followed by cooling and dilution with water to 500 ml.

Summary

1. A method has been evolved for the determination of small amounts of 3-amino-4-hydroxyphenylarsonic acid in acetarsol.

2. The official limit test has been examined by photoelectric methods and the actual limit determined. A more satisfactory test is proposed.

My thanks are due to Mr. Bell for the spectrophotometric absorption curves, to Dr. Hersant for helpful criticisms and to the Directors of May and Baker Limited for permission to publish this paper.

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SYNTHETIC COMPOUNDS POSSESSING CORTICOSTERONE-LIKE ACTIVITY

By R. A. Khan and W. H. Linnell

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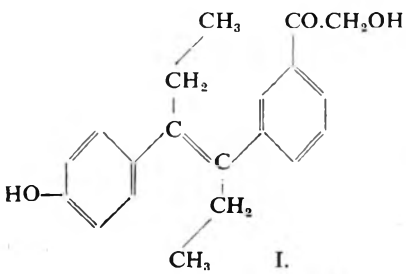
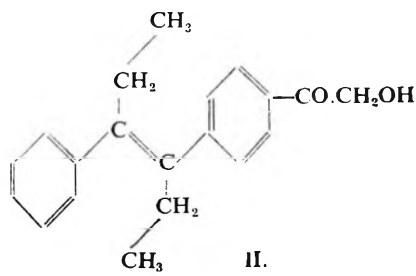
It has been shown by Linnell and Roushdi[1] that 4-hydroxy-3′-(ω-hydroxyaceto)α:β-diethylstilbene (I) possesses approximately 1/200th the activity of desoxycorticosterone, and also that benzoyl carbinol $C_6H_5.COCH_2OH$ exhibits small cortical activity whilst *p*-hydroxybenzoyl carbinol, $OH.C_6H_4.CO.CH.OH$ is completely devoid of activity. It was, therefore, decided to prepare 4-(ω-hydroxyaceto)α:β-diethylstilbene (II) and see if the absence of the hydroxy group in the stilbene compound would enhance activity.



I.



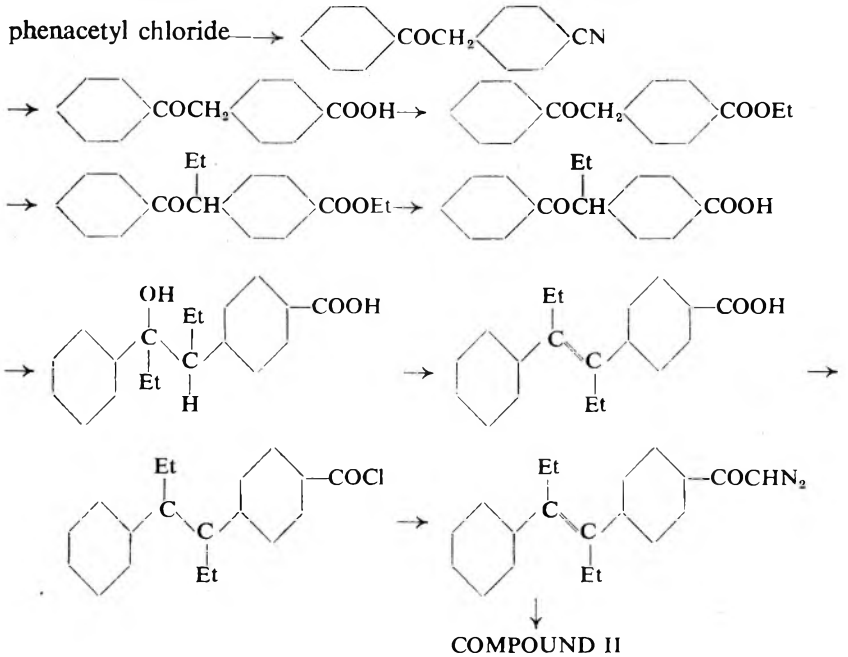
II.

The keto-alcohol group in the new compound is in the 4-position instead of the 3- and this may also have the effect of increasing activity, as in the synthetic hormones derived from the diethylstilbene molecule the 4-position seems to afford the more active compounds.

The compound in question 4-(ω-hydroxyaceto)α:β-diethylstilbene was prepared according to the scheme on page 231.

4-Cyanodesoxybenzoin was obtained by treating *p*-cyanophenacetyl chloride with benzene in the presence of aluminium chloride according to the method of Allen and Barker[2] for preparing desoxybenzoin. The product was hydrolysed, by boiling with sulphuric acid, to 4′-carboxydesoxybenzoin, the ethylation of which at the α-position was achieved after a preliminary esterification and the α-ethyl ester was then saponified by alcoholic caustic potash to give 4-carboxy-α-ethyldesoxybenzoin. By treatment with ethyl magnesium iodide, 3-phenyl-4-(*p*-carboxyphenyl)-hexane-3-ol was obtained which was not isolated in a pure state. This compound was dehydrated by heating with iodine when the 4-carboxy-α:β-diethylstilbene obtained was converted into 4-(ω-hydroxyaceto)-α:β-diethylstilbene by the method of Steiger and Reichstein[3] in which the appropriate acid chloride was treated with diazomethane and the diazoketone so produced decomposed with 2N sulphuric acid. 4-(ω-hydroxyaceto)-α:β-diethylstilbene was obtained in 40 per cent. yield as a bright yellow viscid oil boiling at 175° to 180°C. at 5 mm. Hg.

Benzyl cyanide—→*p*-nitrobenzyl cyanide—→*p*-nitrophenylacetic acid —→*p*-aminophenylacetic acid—→*p*-cyanophenylacetic acid—→*p*-cyano-



COMPOUND II

β-Naphthaleneacetyl carbinol was prepared by a similar method from β-naphthaleneacetic acid and was obtained as a low-melting solid, soluble in boiling water.

Preliminary pharmacological tests, for which we are indebted to Professor Buttle and Dr. Dyer of the Pharmacological Department of this School, indicate that 4-(ω-hydroxyaceto)α:β-diethylstilbene (II) is less active than its corresponding 4′-hydroxy derivative. The naphthaleneacetyl carbinol was found to be inactive at the dose levels used.

This work is being extended to cover other derivatives of naphthalene and hydroxyaceto derivatives of such other systems as may be in any way considered as fragments of the cyclopentenophenanthrene nucleus, including a thorough exploration of the indene skeleton from this point of view.

Experimental

4-*Cyanodesoxybenzoin.*—$C_6H_5.CO.CH_2.C_6H_4.CN$

p-Nitrobenzyl cyanide was obtained in 57 per cent. yield, converted into the corresponding acid (yield 90 per cent.) which was then reduced to *p*-aminophenylacetic acid (yield 85 per cent.) according to the method of Robertson[4,5,6]. This compound was converted into *p*-cyanophenylacetic acid by following the process of Jaeger and Robinson[7] when a 59 per cent. yield was realised. 5 g. of phosphorus trichloride was added to 10 g. of dry *p*-cyanophenylacetic acid and the mixture heated on a water-bath till completely liquid. While still warm, 50 ml. of dry benzene

was added and the benzene solution decanted on to 10 g. of anhydrous aluminium chloride. When the spontaneous reaction had subsided the mixture was refluxed for 1 hour, cooled and poured into a solution of 25 ml. of concentrated hydrochloric acid in 500 ml. of cold water. The benzene layer was separated, the aqueous portion was washed with benzene and the washings combined with the benzene solution. The solvent was removed by distillation and the yellow sticky residue was crystallised from a small quantity of alcohol (97 per cent.) when 4′-cyanodesoxybenzoin was obtained as light yellow crystals melting at 105° to 106°C. Yield 5·7 g. (41 per cent.). Found : C, 80·1 ; H, 5·0 ; N, 6·33 per cent. ; $C_{15}H_{11}OH$ requires C, 81·4 ; H, 4·9 ; N, 6·35 per cent.

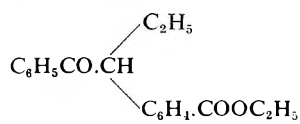
4′-*Carboxydesoxybenzoin.*—$C_6H_5.CO.CH_2.C_6H_4.COCH$

1 g. of 4′-cyanodesoxybenzoin was refluxed with 25 ml. of glacial acetic acid, 25 ml. of water and 25 ml. of concentrated sulphuric acid for 5 hours and poured into an equal volume of cold water. The thick white precipitate was collected, washed with cold water and crystallised from 60 per cent. acetic acid. After purification by animal charcoal and recrystallisation from 60 per cent. acetic acid, 4′-carboxy-desoxybenzoin was obtained as shining white flakes melting at 209°C. (decomp.). Yield 0·75 g. (63 per cent.). Found : C, 74·3 ; H, 5·1 ; per cent. $C_{15}H_{12}O_3$ requires C, 75·0 ; H, 5·0 per cent.

4′-*Carbethoxydesoxybenzoin.*—$C_6H_5.CO.CH_2.C_6H_7.COOC_2H_5$

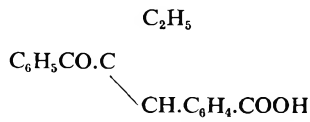
4′-Carboxydesoxybenzoin was esterified by bubbling hydrogen chloride through an alcoholic solution of 3 g. of the acid and refluxing the mixture at the same time when a quantitative yield of 4′-carbethoxydesoxybenzoin was obtained as pale shining flakes melting at 106°C. Found C, 75·1 ; H, 6·02 per cent. ; $C_{17}H_{16}O_3$ requires C, 76·1 ; H, 5·97 per cent.

4′-*Carbethoxy-α-ethyldesoxybenzoin.*—



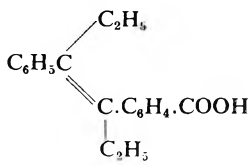
2·5 g. of 4′-carbethoxydesoxybenzoin was dissolved in 10 ml. of boiling absolute alcohol and to the boiling solution 0·5 g. of sodium in 5 ml. of absolute alcohol and 3·3 g. of ethyl iodide were added ; the mixture being heated until neutral to litmus. A further quantity of 0·5 g. of sodium in 5 ml. of absolute alcohol and 3·3 g. of ethyl iodide was added and the mixture again refluxed until neutral. After cooling and dilution with water, the alcohol was removed under reduced pressure. The oily suspension was extracted with ether and the ethereal solution well washed with water, 5 per cent. sodium thiosulphate solution and water. After drying overnight the ether was removed by distillation and 4′-carbethoxy-ethyldesoxybenzoin was obtained as a bright yellow oil which solidified to a glassy substance. Yield 2·1 g. (90 per cent.). Found : C, 77·0 ; H, 6·79 per cent. ; $C_{19}H_{20}O_3$ requires C, 77·02 ; H, 6·75 per cent.

4′-Carboxy-α-ethyldesoxybenzoin.—



2·1 g. of 4′-carbethoxy-α-ethyldesoxybenzoin was saponified with 110 ml. of N/10 alcoholic potassium hydroxide solution. The crude oily substance was crystallised from 70 per cent. acetic acid, when fine white crystals of 4′-carboxy-α-ethyldesoxybenzoin melting at 126° to 127°C. were obtained. Yield 1·42 g. (75 per cent.). Found : C, 75·98 ; H, 5·95 per cent. : $C_{17}H_{16}O_3$ requires C, 76·11 ; H, 5·97 per cent.

4′-Carboxy-α:β-diethylstilbene.—



A cold ethereal solution of ethyl magnesium iodide (prepared by adding 1·65 g. of ethyl iodide to 0·25 g. of dry magnesium in ether) was added slowly with frequent shaking to a solution of 2·7 g. of 4′-carboxy-α-ethyldesoxybenzoin in dry ether. The reaction mixture, protected from moisture, was left overnight and then refluxed for 3 hours. The resulting product was decomposed with ice and dilute sulphuric acid, and the product worked up in the usual way after which the oily residue was heated on a water-bath with 1 to 2 per cent. of iodine under an air condenser for 1 hour. The residue, dissolved in ether, was washed with water and 5 per cent. sodium thiosulphate solution and dried overnight. The solvent was removed by distillation and 2 g. of a dark brown oil was obtained. From boiling 90 per cent. acetic acid, on scratching the sides of the vessel and standing, it gave a white powder melting at 95° to 100°C. Titration with N/125 sodium hydroxide indicated a purity of 96·1 per cent. Its acid chloride prepared as described below gave a crystalline 4-amido-derivative melting at 94°C. Found : N, 4·79 per cent. ; $C_{19}H_{11}ON$ requires N, 5·01 per cent.

4-(ω-Hydroxyaceto)-α:β-diethylstilbene.—$C_{18}H_{19}.COCH_2OH$

1·5 g. of 4-carboxy-α:β-diethylstilbene was converted into its acid chloride by boiling with 5 ml. of dry benzene and 5 ml. of thionyl chloride. The acid chloride in dry ether was treated with an ethereal solution of diazomethane (prepared from 10 g. of nitrosomethylurea) and the mixture kept at 6°C. for 1 hour and then allowed to stand overnight at room temperature. After removing the excess of diazomethane and the solvent, the diazoketone, obtained as a dark oil, was dissolved in 10 ml. of dioxan and, on addition of 15 ml. of 2N sulphuric acid, nitrogen was liberated during about half an hour, after which the reaction mixture was warmed to 40° to 45°C. when no more gas was evolved. It was then diluted with water and extracted with ether. The ethereal solution was washed

with water and a small quantity of 1 per cent. sodium bicarbonate solution. After drying the ethereal solution, ether was removed by distillation and the dark oil distilled under vacuum. 4-(ω-Hydroxyaceto)-α:β-diethylstilbene was obtained as a yellow oil boiling at 175° to 180°C. / 5 mm.Hg. Yield 0·8 g. (40 per cent.). Found C, 78·8 ; H, 7·5 per cent. : $C_{20}H_{22}O_2$ requires C, 81·2 ; H, 7·6 per cent. It was soluble in ether, alcohol, acetone, benzene, chloroform and toluene, but insoluble in water. It reduced cold ammoniacal silver nitrate solution and gave an osazone with 2:4-dinitrophenylhydrazine, which, on recrystallisation from toluene, melted at 115°C. Found : N, 17·5 per cent. : $C_{32}H_{28}O_8N_8$ requires N, 17·18 per cent.

β-Naphthaleneacetyl carbinol.—$C_{10}H_7.CH_2.CO.CH_2.OH$

An ethereal solution of β-naphthaleneacetyl chloride obtained from 1 g. of β-naphthaleneacetic acid was treated with diazomethane as described earlier. The resulting diazoketone was dissolved in 10 ml. of dioxan, decomposed with 5 ml. of 2N sulphuric and worked up as stated previously to give 0·71 g. (63 per cent.) of β-naphthaleneacetyl carbinol melting at 90° to 93°C. as light yellow crystals from 97 per cent. alcohol. Found : C, 75·32; H, 5·91 per cent.; $C_{13}H_{12}O_2$ requires C, 78·00; H, 6·00 per cent.

It was soluble in most organic solvents and also in boiling water. It reduced ammoniacal silver nitrate solution in the cold. With 2 : 4-dinitrophenylhydrazine it gave the corresponding hydrazone melting at 24°C. The hydrazone on analysis gave N, 14·0 per cent. : $C_{19}H_{16}O_5$ requires N, 14·7 per cent.

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SYNTHESIS OF MONOMERIC FORMS OF α-HYDROXY-β-METHOXYPROPIONALDEHYDE AND 1:3-DIMETHOXY-PROPANONE-2

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The compounds concerned in this communication are required for the synthesis of methylated hexoses which could not possess a pyranose structure.

α-Hydroxy-β-methoxypropionaldehyde had not been obtained in the monomeric form, although the dimeride was prepared by Fisher and Bear[1] and proved to be a crystalline substance melting at 120° to 121°C. which on heating with pyridine suffered rearrangement into the monomethyl ether of dihydroxyacetone. Alkaline condensation of this dimeride gave rise to sugars with branched chains.

The monomeric form was prepared by the following series of reactions:

$$CH_2OH.CHOH.CH_2OH \rightarrow CH_2Cl.CHOH.CH_2OH \rightarrow$$
$$CH_2OCH_3.CHOH.CH_2OH \rightarrow CH_2OCH_3.CHOH.CHO$$

The α-monomethylether of glycerol was obtained by the gradual addition of a methyl alcoholic solution of glycerol-α-monochlorohydrin to a solution of sodium methylate, the mixture being refluxed until separation of sodium chloride ceased: it was obtained as a syrup boiling at 135° to 136°C./40 mm. Hg. pressure. The oxidation was attempted *via* the Oppenhauer[2] reaction using aluminium tertiary butoxide as this method was successful in the oxidation of cholesterol to cholestenone as applied by Adkins and Cox[3]. The conditions of Adkins and Cox were used, but a very poor yield was obtained.

Fenton and Jackson[4] originated the hydrogen peroxide-ferrous sulphate oxidation of polyhydric alcohols. This reagent appears to be specific for the primary alcoholic groups, no instance of ketone formation being reported. It has been successfully used for the oxidation of ethylene glycol to glycol aldehydes, mannitol to mannose[5] and glycerol to glyceraldehyde. After numerous trials satisfactory conditions were established for the oxidation of the α-monomethylether of glycerol, and the product on isolation gave a positive test with Schiff's reagent, reduced Fehling's and ammoniacal silver nitrate solutions, gave an immediate precipitate with 2:4-dinitrophenylhydrazine and a solid bisulphite compound. The product obtained with 2:4-dinitrophenylhydrazine, melted at 234° to 236°C. (with decomposition) and gave analytical figures in agreement with those of the osazone of α-hydroxy-β-methoxypropionaldehyde. The formation of the osazone proves the

monomeric character of the substance, and this is further supported by the fact that the hydrazone could not be isolated, the dimeric forms yielding hydrazones under similar conditions.

The compound could not be further purified by distillation under very low pressure as polymerisation then occurred. The syrup itself darkens slowly on exposure to air, and hence it was always used immediately after preparation. Reeve[6] had previously observed that the monomeric form of glyceraldehyde was a syrup whilst the bimolecular was crystalline.

The synthesis of 1 : 3-dimethoxypropanone-2 was achieved as follows: glycerol → 1 : 3-glycerol-dichlorohydrin → 1:3-dimethoxypropanol-2 → 1:3-dimethoxypropanone-2. Previous methods of preparation included the ketonic hydrolysis of α-γ-dialkoxyacetoacetic esters[7,8,9] and the distillation of calcium salts of the alkoxyacetic acids. Methylation of symmetrical dichloroacetone with sodium methylate failed owing to the polymerisation of the product during the isolation.

Eventually the required compound was achieved by acid chromate oxidation, at 20° to 23°C., of 1 : 3-dimethoxypropanol-2 (b.pt. 58° to 60°C./12 mm. Hg. pressure), which was obtained from glycerol-1 : 3-dichlorohydrin (b.pt. 70° to 72°C./14 mm. Hg. pressure) by the gradual addition of its solution in methyl alcohol to a solution of sodium methoxide. The isolated product was finally fractionated, and the portion boiling at 42°C./22 mm. Hg. pressure answered all the requirements. It gave positive colour tests with sodium nitroprusside, *m*-dinitrobenzene and 3 : 5-dinitrobenzoic acid characteristic of ketones; it reduced Fehling's solution and ammoniacal silver nitrate solution and gave a 2 : 4-dinitrophenylhydrazone melting at 112° to 113°C., which gave correct analytical figures. The ketone itself gave the analytical figures required, and hence the structure was confirmed.

Experimental

α-Hydroxy-β-methoxypropionaldehyde $CH_2OCH_3.CHOH.CHO$.—The oxidation of glycerol-α-monomethylether boiling at 136°C./40 mm. Hg. pressure, obtained by treatment of glycerol-2-chlorohydrin (b.pt. 104° to 108°C./9 mm. Hg.) with sodium methoxide, was attempted (A) with aluminium tertiary butoxide. 100 g. of the monomethylether was placed in a dry 5-l. flask provided with a reflux condenser and a drying tube together with 750 ml. of pure dry acetone and 1 l. of pure benzene. To this mixture, maintained between 75° and 85°C., a solution of 80 g. of aluminium tertiarybutoxide in 500 ml. of dry benzene was added in one portion. The mixture turned milky and was refluxed for a total period of 8 hours. After cooling, 200 ml. of water, 500 ml. of 10 per cent. sulphuric acid and a further 1·5 l. of water were added successively and the whole allowed to separate. The separated aqueous layer was extracted with benzene, and the combined benzene solutions were dried over anhydrous sodium sulphate. The aqueous layer was neutralised with barium carbonate and fractionated, but the isolation of the desired

product was not achieved. The higher boiling fraction, collected between 85° and 95°C., was saturated with salt and extracted many times with chloroform. On removal of the chloroform little residue was obtained. The aqueous solution saturated with salt was therefore extracted with ether, and from this ethereal solution 2 ml. of a brown syrup was isolated which gave a positive test with Schiff's reagent.

(B) As a result of the above failure the oxidation was attempted with Fenton's reagent. The α-monomethyl ether was diluted with an equal bulk of water and mixed with an aqueous solution of 2·5 g. of ferrous sulphate. Hydrogen peroxide (20 vol.) 112·5 ml. was gradually added during half an hour, or until the reaction ceased, the flask being cooled between the separate additions. After standing for 2 hours excess of finely powdered barium carbonate was added, the mixture filtered and the clear and colourless filtrate concentrated under reduced pressure at a temperature not higher than 30°C. The residue was extracted with absolute alcohol, ether being added to the filtered alcoholic solution until no more solid separated. The clear alcohol-ether solution was concentrated under reduced pressure at 20°C., the residue being a colourless syrupy liquid which responded to qualitative tests for aldehydes. In all 321 g. was prepared.

α-Hydroxy-β-methoxypropionaldehyde-2:4-*dinitrophenylosazone.* — A solution of the above syrup (2 g.) in 5 ml. of absolute alcohol was added to a solution of 2:4-dinitrophenylhydrazine (2 g.) and 1 ml. of concentrated sulphuric acid in 25 ml. of absolute alcohol. An immediate precipitation occurred and the mixture was warmed at 66° to 70°C. for 20 minutes. On cooling, the fine crystals which separated were collected, washed, dried and recrystallised twice from alcohol. They appeared as fine orange needles melting at 234° to 236°C. after drying *in vacuo.* Found: N, 24·8 per cent.; $C_{16}H_{14}O_9N_8$ requires N, 24·24 per cent.

1:3-*Dimethoxypropanol* $(CH_3OCH_2)_2CHOH$.—Glycerol-1:3-dichlorohydrin, 100 g. dissolved in 120 ml. of methyl alcohol, was slowly added with vigorous shaking to the solution obtained by the addition of 40 g. of sodium to 560 ml. of methyl alcohol. After standing for 3 hours the reaction was completed by refluxing for 6 hours. The product was isolated in the usual manner and purified by fractional distillation. The fraction boiling at 55° to 60°C./12 mm. Hg. pressure consisted mainly of the required substance and was redistilled collecting the fraction boiling at 58° to 60°C./12 mm. Hg. pressure.

1:3-*Dimethoxypropanone*-2 $(CH_3OCH_2)_2C:O$.—To a mixture of 50 g. of 1:3-dimethoxypropanol-2, 75 ml. of water and 42 g. of powdered sodium dichromate, 52 g. of sulphuric acid diluted with 29 ml. of water was added at intervals of 10 minutes during 5 hours whilst stirring vigorously and maintaining the temperature between 22° and 23°C. The stirring was continued for a further 3 hours when tests for hydrogen peroxide became negative. The mixture was extracted with ether, the

ethereal extracts being washed with sodium bicarbonate solution, with water, and then dried over anhydrous sodium sulphate. On removal of the ether 10 g. of a colourless syrup remained. After precipitation of chromium salts with sodium bicarbonate a further 15 g. of colourless syrup was obtained from the mother liquors by extraction with absolute alcohol. The combined syrups were then fractionated under reduced pressure when a main fraction boiling at 40° to 42°C./22 mm. Hg. pressure was obtained. On redistillation this fraction yielded 20 g. of 1:3-dimethoxypropanone-2. Yield, 40 per cent. Found: C, 49·76; H, 8·44 per cent.; $C_5H_{10}O_3$ requires C, 50·85; H, 8·47 per cent. The product gave positive qualitative reactions of a ketone.

1:3-*Dimethoxypropanone*-2-2′:4′-*dinitrophenylhydrazone*.

$(CH_3O.CH_2)_2C:N.NH.C_6H_3(NO_2)_2$.—A solution of 2 g. of 1:3-dimethoxypropanone-2 in 5 ml. of absolute alcohol was mixed with a filtered solution of 2:4-dinitrophenylhydrazine (2 g. in 30 ml. of absolute alcohol plus 1 ml. of concentrated sulphuric acid). The mixture was warmed and, on cooling, yellow needle-shaped crystals separated. After two recrystallisations from boiling alcohol and drying *in vacuo* the melting point was found to be 112° to 113°C. Found: C, 44.66; H, 4·75; N, 18·8 per cent.; $C_{11}H_{14}O_6N_4$ requires C, 44·30; H, 4·70; N, 18·79 per cent.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Alkaloids of the Australian Rutaceæ. G. K. Hughes, F. N. Lahey, J. R. Price and L. J. Webb. (*Nature*, 1948, **162**, 223.) As part of a survey of the occurrence and pharmacology of alkaloids in the Australian flora, three Queensland rain-forest species have been examined. The bark of *Melicope fareana* F. Muell. (bark) contained melicopine ($C_{17}H_{15}O_5N$), pale yellow needles, m.pt. 178·5° to 179·5°C. melicopidine ($C_{17}H_{15}O_5N$), pale yellow prisms, m.pt. 121° to 122°C. melicopicine, yellow prisms, m.p.t. 131° to 132°C. acronycidine ($C_{15}H_{15}O_5N$), colourless needles, m.pt. 136·5° to 137·5°C. While the leaves of *M. fareana* also contained melicopine, melicopidine and melicopicine, acronycidine was absent. Another colourless alkaloid was present but has not yet been obtained pure. The bark of *Acronychia baueri* Schott contained acronycine ($C_{20}H_{19}O_3N$), pale yellow needles, m.pt.174° to 175°C. melicopine, melicopidine and acronycidine, while melicopicine was also isolated from the leaves. The bark of *Evodia xanthoxyloides* F. Muell contained evoxanthine ($C_{16}H_{13}O_4N$), pale yellow needles, m.pt. 217° to 218°C. and melicopidine. It has been established that the five yellow alkaloids are N-methyl acridones. Acronycidine (colourless) is considered to be a quinoline derivative related to skimmianine.

F. H.

ANALYTICAL

Arsenic and Iron, Volumetric Microdetermination of. G. F. Smith and J. S. Fritz. (*Anal. Chem.*, 1948, **20**, 874.) The equivalence point for the potentiometric titration of 0·001N solutions of ferrous iron, oxalic and arsenite ions in 2F perchloric acid solutions by oxidation using 0·001N solutions of perchloratoceric acid in the same acid medium covers the range 0·95 to 1·5 volts, and the ratio of change in potential to oxidant addition is greater at 1·23 volts. Hence, by using a redox indicator, ferrous nitrophenanthrolinium ion (nitroferroin) having a transition potential of 1·23 volts in 2F perchloric acid the more laborious potentiometric titration procedure is avoided. The colour change is red to faint blue. Results are accurate to ±0·5 per cent. In the case of iron, results obtained by the above method and the spectrophotometric determination are of comparable accuracy.

E. N. I.

Capsaicin, Assay of. J. Büchi and F. Hippenmeier. (*Pharm. Acta Helvet.*, 1948, **23**, 327.) In testing capsicum preparations by taste, it is found that the sensitivity of different individuals shows very great variations, the sensitivity of the tongue rapidly decreases, and recovery of the sensitivity requires about 3 hours. A new method of assay is based on a study of the properties of pure capsaicin. Chemically, capsaicin is a vanillylamine acylated with a decylenic acid. The most important groupings are a double bond, an acid amide grouping, a free phenolic hydroxyl, and a methylated phenolic hydroxyl group. Attempts to base a method of assay on the reactions of one of these groupings were unsuccessful for various reasons. A method based on the reducing power of capsaicin is as follows: about 10 mg. of

capsaicin is dissolved, with gentle warming, in 45 ml. of 0·1N sodium hydroxide, and made up to 50 ml. with the alkali; 5 ml. of this solution is treated with 3 ml. of reagent (3 g. of pure phosphomolybdic acid in 100 ml. of water), and, after standing for 1 hour, is examined colorimetrically in a 0·5 cm. cell, using filter S72. A standardisation curve is prepared with pure capsaicin. This method has not yet been extended to the assay of capsicum itself. G. M.

Capsaicin in Drugs, Determination of. J. Büchi and F. Hippenmeier. (*Pharm. Acta Helvet.*, 1948, **23,** 353.) The method is based on the colorimetric reaction for capsaicin previously described by the authors (*Pharm. Acta Helvet.*, 1948, **23,** 327). Details are as follows: 5g. of powdered capsicum is shaken for 30 minutes with 50 ml. of dilute alcohol, and the mixture is filtered into a 250-ml. beaker, the filter being washed with 20 ml. of dilute alcohol. After the addition of 15 ml. of 0·5N sodium hydroxide to the filtrate, the mixture is warmed on the water-bath, with occasional stirring, for about 1 hour until the alcohol is removed and the mixture does not froth on stirring. The solution is cooled and treated with 2N hydrochloric acid, added drop by drop, until it does not turn thymol blue paper blue but still reacts alkaline to litmus paper. This solution, which may be slightly turbid, is then shaken out with 50, 25, and 25 ml. of ether. The combined clear ether extracts are dried with sodium sulphate, and the ether is removed on the water-bath, the residue being dried for 30 minutes in a current of air. The residue is dissolved by gentle heat in 20 ml. of (exactly) 0·1N sodium hydroxide, used in two portions, and the solution is strained and made up to 25 ml. with the alkali. This solution should be clear or at most slightly opalescent. 5-ml. portions are transferred to two 20-ml. measuring flasks, and to a 20-ml. stoppered tube. To each of the flasks, is added 3 ml. of reagent (3 g. of phosphomolybdic acid in water to 100 ml.), while 3 ml. of water is added to the tube. After 1 hour, both flasks are made up to the mark with alcohol (95 per cent.), and shaken vigorously, final adjustment of the volume being made after cooling. The contents of the tube are diluted similarly to 20 ml. The extinction of the liquids in the flasks is then measured against that in the tube, using a 0·5 cm. cell and filter S72. The amount of capsaicin in mg./5ml. of the 0·1N sodium hydroxide solution is then equal to the extinction co-efficient $\times \frac{2.5}{1.100}$ The figures obtained from a number of samples of the official Swiss drug (*Capsicum annuum*) range from 0·122 to 0·304 per cent. One sample of cayenne showed 0·415 per cent. In applying the method to the tincture, 50 g. of the latter is shaken out with 50 ml. of light petroleum the extract being washed with 3 quantities, each of 25 ml. of dilute alcohol. The combined alcoholic solutions are combined with the original tincture which has been extracted with light petroleum and, after the addition of 15 ml. of 0·1 N sodium hydroxide, the method is continued as before. For concentrated fluid extract of capsicum (oleoresin of capsicum), 1 g. of the material is dissolved in 40 ml. of light petroleum and this solution is shaken out 3 times with 20 ml. quantities of dilute alcohol. The combined alcoholic solutions are treated as before. G. M.

Extracts, Determination of Moisture in, by Infra-red Heating. M. Bouchardy and A. Mirimanoff. (*Pharm. Acta Helvet.*, 1948, **23,** 321.) The determination of the moisture content of dry extracts by heating in an oven is unsatisfactory, as constant weight is never obtained.

The use of infra-red rays is quicker and more satisfactory. The lamp, placed at a distance of 37 cm. from the material, raises its temperature to about 62°C., and drying is generally complete in 1 to 2 hours, when the extract is spread out in a layer of 1 to 2 mm. thickness and 30 sq. cm. area. Even in this case loss of weight continues after all the moisture has been removed. The presence of residual traces of water may be detected by using the moisture-detecting powder recently described (Baymond, *Pharm. Acta Helvet.*, 1948, **23,** 207; *J. Pharm. Pharmacol.*, 1949, **1,** 44), the extract being placed in a small crucible heated in an oil-bath at 130°C., and covered with a watch glass which has a coating of the water-detecting powder. The dry extracts examined contained in general less than 3 per cent. of moisture. Figures of over 10 per cent., sometimes recorded in the literature, are erroneous. G. M.

Glycerol, Colorimetric Method for the Determination of Small Quantities of. V. H. Mikkelsen. (*Analyst,* 1948, **73,** 447.) The method used depends on the fact that glycerol is oxidised by bromine to 1:3-dihydroxyacetone and probably also to glyceraldehyde; addition of concentrated sulphuric acid yields methyl glyoxal which will react with codeine, thymol, resorcinol and β-naphthol to form coloured compounds. Codeine was chosen for the reaction as it gave a blue colour with a characteristic absorption band with a maximum at 6600Å. By systematic variation of (1) the amount of bromine, (2) the glycerol concentration, and (3) the heating time both for the oxidation and for the codeine reaction, a procedure was obtained which gave reproducible results and proportionality between the extinction coefficient and the glycerol concentration. Details of the final colorimetric method are given, together with details of the method as applied to the determination of glycerol in a morphine injection. In place of saturated bromine water an aqueous solution of potassium bromate and bromide containing an equal volume of 2N hydrochloric acid was used. R. E. S.

Hyoscine and Hyoscyamine, Separation of, and the Alkaloidal Assay of *Duboisia* spp. E. M. Frautner and M. Roberts. (*Analyst,* 1948, **73,** 140.) Hyoscine and hyoscyamine, including atropine, occur together in several solanaceous plants, and are generally determined together as total alkaloids, and calculated in terms of hyoscyamine. Two methods of separation of the two alkaloids are described, the first, an approximately quantitative separation of the alkaloids by chromatographic adsorption on activated silica, and subsequent fractional elution, and the second, the separation and identification of the components of a mixture of alkaloids by fractional precipitation of their picrates. Hyoscine and hyoscyamine are both strongly adsorbed on a silica column from benzene solution, and are separated by elution with absolute alcohol. The hyoscine is removed rapidly but the hyoscyamine only slowly. By adding a trace of dimethylamino-azo-benzene (butter yellow, dimethyl yellow, C.I.19) to the benzene the alkaloids can readily be detected on the column. The dye is only weakly adsorbed by the silica, giving a brilliant red colour, except where the alkaloids are preferentially adsorbed; here a yellow band of unadsorbed dye is left. The dye is quickly removed by elution with ether or absolute alcohol, but if these solvents are then replaced by benzene or, better, light petroleum, containing a little of the dye, the adsorption zones reappear. It can then be seen, which zones have been eluted, separated or spread. The efficiency of the separation of the alkaloids is dependent on the dimensions of the silica column, the authors used a column 12 cm. x 1 cm. The best results are obtained when the ratio of the amount of hyoscine to

that of hyoscyamine lies between the limits 1 : 4 and 6 : 1. The fractions obtained are pure enough to give crystalline picrates, even when only 1 or 2 mg. of alkaloid is present. The picrates are prepared by the addition of a dilute solution of picric acid, followed by crystallisation from chloroform, and are readily identified by their m.pt., hyoscine picrate usually melts at the correct temperature, 187° to 188°C., but the hyoscyamine picrate is frequently less pure and may melt several degrees lower than the correct value, 165° to 166°C. The separation of hyoscine and hyoscyamine picrates is too slow to replace the better and more rapid fractionation which can be achieved with a silica column, it is, however, useful if it is necessary to establish the identity of the main alkaloid present in the hyoscyamine fraction. Using these methods to assay a sample of *Duboisia myoporoides* the authors found that the leaves contained about 2 per cent. of hyoscyamine, 0·7 per cent. of hyoscine, and 0·2 per cent. of other alkaloids. Detached leaves of *Atropa belladonna*, starved to the point of proteolysis, assayed by similar methods, showed that the hyoscyamine content remained unchanged, and that neither hydrolysis nor demethylation takes place to any appreciable extent. L. H. P.

Inositol, Chemical Determination of. P. Fleury and A. Leconles. (*C. R. Acad. Sci., Paris,* 1948, **227,** 691.) The method depends upon measuring the volume of carbon dioxide released when inositol is treated with periodic acid. Though several days are necessary for complete evolution, the majority is released within a short time and this quantity is proportional to the concentration of inositol. Interfering substances are first destroyed by treating with magnesia at 100°C. Experiments with known concentrations of inositol added to urine showed that an accuracy of about ±2·5 per cent. was normally obtained, with a maximum error of ±10 per cent. J. W. F.

Iodine in Organic Compounds, Micro-determination of. R. Grangaud. (*Ann. pharm. Franc.,* 1948, **6,** 212.) The method is based on the reaction of iodide, in solution, with silver iodate, forming soluble iodate. The organic compound is decomposed in a tube in a current of oxygen, the gases evolved being passed over heated platinum foil. The iodine is absorbed in 0·75 ml. of 0·2N sodium hydroxide containing 5 drops of 30 per cent. hydrogen peroxide. After the combustion, the hydrogen peroxide is destroyed by heating for 5 minutes on the water-bath, methyl red is added, and the liquid is adjusted to a *p*H of about 5. It is then cooled in ice and treated with about 50 mg. of solid silver iodate, and shaken vigorously for 1 minute. The mixture is filtered by syphoning (Pregl method) through a sintered glass micro-filter covered with a layer of asbestos. The filter is washed with 0·5 ml. of dilute alcohol, then twice with alcohol (95 per cent.). The filtrate is diluted with water to about 30 ml. and treated with a few crystals of potassium iodide and 1 ml. of 10N sulphuric acid. After 1 minute, the liberated iodine is titrated with 0·02N iodine, using starch as indicator. Owing to the formation of traces of nitrous acid, the method must be modified for the assay of nitrogenous substances or for liquids which are sealed into a capillary in presence of a crystal of ammonium nitrate. In this case the hydrogen peroxide is omitted, and after the combustion the liquid in the absorber is treated with 5 drops of sodium bisulphite solution and 0·2 ml. of N sulphuric acid. After heating on the water-bath for 10 minutes to destroy the nitrous acid, the liquid is cooled and treated with 5 drops of hydrogen peroxide and 0·2 ml. of N sodium hydroxide. The assay is then continued as before. G. M.

Iron, Colorimetric Determination of, with *iso*Nitrosodimethyldihydroresorcinol. S. C. Shome. (*Anal. Chem.*, 1948, **20**, 1205.) A spectrophotometric study of the colorimetric determination of iron (ferrous or ferric) has been made using the blue colour given with *iso*nitrosodimethyldihydroresorcinol. The effect of concentration of the reagent was studied together with problems of iron concentration, colour stability, and interference of other ions. The colour formed was found to be stable and both ferrous and ferric iron reacted; iron could thus be determined in the presence of comparatively large amounts of nickel, cobalt, phosphate, arsenate, fluoride, oxalate, citrate, tartrate, borate, and perchlorate in slightly acid medium. Sensitivity measurements indicated that iron can be detected with this reagent to an extent of 1 part in 50,000,000 parts of solution. Analyses of the purified iron-*iso*nitrosodimethyldihydroresorcinol complex showed that an iron atom combines with three molecules of the organic reagent; the complex retained two molecules of water even after drying in a vacuum desiccator over sulphuric acid for a number of days. R. E. S.

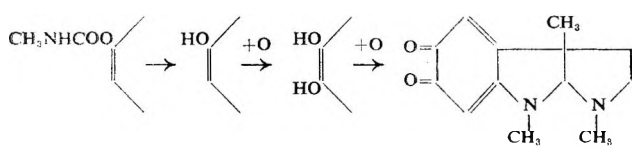
Œstrogens, Synthetic, Colorimetric Determination of. A. Carayon-Gentil and M. J. Cheymol. (*Ann. pharm. Fr.*, 1948, **6**, 129.) Methods are given for the determination of stilbœstrol, hexœstrol and dienœstrol in tablets and oily solutions. *Extraction of tablets:* the finely powdered material, corresponding to 1 to 3 mg. of œstrogen, is shaken mechanically with two quantities of ethyl acetate, 40 ml. in all, each shaking being continued for 1 hour. The mixture is filtered, and the residue washed with water. After evaporation of the solvent, the residue is taken up in methyl alcohol. If the tablets are prepared with esters of the œstrogens, a saponification must follow the extraction. *Oily solutions:* 1 ml. of solution, containing 1 to 2 mg. of œstrogen, is refluxed with 15 ml. of 5N methyl alcoholic solution of potassium hydroxide until saponification is complete. After diluting with 4 volumes of water, unsaponifiable matter is removed by shaking twice with light petroleum, and the aqueous phase is acidified and extracted with ether. The ethereal solution is washed with sodium carbonate solution (1 per cent.) then with water. The ether is removed by evaporation, and the residue is taken up in methyl alcohol so that 1 ml. of the solution corresponds to 50 to 100 μg of œstrogen. For dienœstrol a different method is employed: 1 to 2 ml. of solution, containing 2 to 5 mg. of dienœstrol, is shaken mechanically for 30 minutes with 20 ml. of methyl alcohol, and the extraction is repeated with a further quantity of methyl alcohol. The combined methyl alcoholic solution is filtered and used for the determination. *Determination of dienœstrol.* A coupling method is used. One ml. of the methyl alcoholic solution is mixed with 8 ml. of borate buffer solution, *p*H 12 (20 g. of boric acid and 280 ml. of N sodium hydroxide per l.) heated for 6 minutes at 80°C., then cooled to 20°C. It is then treated with 2ml. of diazotised sulphanilic acid solution and, after 5 minutes, the colour is compared with that obtained from a standard solution of dienœstrol. Dienœstrol from oily solutions may give a final solution which is slightly turbid. It can be cleared by adding 1 or 2 drops of a 10 per cent. solution of calcium chloride. G. M.

Pentaerythrityl Tetranitrate and Glyceryl Trinitrate, Determination of. J. Allert. (*Dansk Tidsskr. Farm.*, 1948, **22**, 188.) By saponification of alkyl nitrates with alkali, nitrites are formed and may be determined colorimetrically with e.g. procaine and α-napthylamine. This method is

however not satisfactory for pentaerythrityl tetranitrate. After *trans*-esterification with sulphuric acid, the nitric acid may be determined with phenoldisulphonic acid, but the brucine method does not give satisfactory results. Details are as follows: 1 ml. of a solution (10 to 100 mg./l.) in glacial acetic acid is treated with 2 ml. of phenoldisulphonic acid and heated for 10 minutes on the water-bath. After the addition of 25 ml. of ammonia solution (25 per cent.), the mixture is made up to 100 ml. The colour is determined photometrically (filter S43) using potassium nitrate as standard. For the determination of pentaerythrityl tetranitrate and glyceryl trinitrate in admixture in tablets the method is as follows: The sample is extracted with 3 ml. of acetone, and rinsed twice with 1 ml. of water. The filtered solution is made up to 100 ml. with water, and the pentaerythrityl tetranitrate which separates out is filtered off. The glyceryl trinitrate content of the solution is determined by the procaine-napthylamine method, and the pentaerythrityl tetranitrate by difference from the total nitrate. G. M.

Persulphates, Iodimetric Titration of. D. J. de Jong. (*Pharm. Weekbl.*, 1948, **83**, 596.) The reaction between persulphate and iodide is slow, but may be accelerated by traces of iron and by warming. The method recommended is as follows: 0·3 g. of the salt is dissolved in 20 ml. of water and treated with 3 g. of potassium iodide and 0·1 g. of ferrous sulphate, followed by 10 ml. of dilute hydrochloric acid and 50 ml. of water. The liberated iodine is titrated with 0·1N sodium thiosulphate until colourless, when starch solution is added, followed by 100 ml. of boiling water. The titration is then continued until the blue colour does not reappear. G. M.

Physostigmine, Photometric Determination of. I. Ehrlés. (*Farm. Revy*, 1948, **47**, 519.) The determination is based on the conversion of physostigmine to rubreserine by way of the following reactions:



The method has been used before, but in the present one somewhat different conditions are employed, with a more closely defined oxidation process. Rubreserine, gives similar, but not identical absorption curves in acid or alkaline solution, with two maxima, as follows:

at *p*H 1·10 { 460 mμ log e = 3·56 ; 295 „ „ 4·06

in 0·02 M sodium hydroxide { 480 „ „ 3·61 ; 298 „ „ 4·11

At *p*H values 2 to 6, intermediate curves are obtained.

The pk_A value for rubreserine was found to be 4·03. The methods employed for the determination of physostigmine salicylate is as follows: 1 to 4 mg. is mixed with 1·0 ml. of a 2 per cent. solution of potassium ferricyanide, 1 ml. of M sodium hydroxide, and made up to 50 ml. with water. After 10 to 15 minutes the extinction is read at 480 mμ. A blank test is also carried out on the reagents. The molar extinction coefficient is between 3900 and 4000. G. M.

ESSENTIAL OILS

Cajuput Oil, Characters of. W. Spoon and W. M. Sesseier. (*Pharm. Weekbl.*, 1948, **83**, 593.) A sample of cajuput oil obtained from England showed the following characters; which are compared with the requirements of the Dutch Pharmacopœia:

	Sample	Dutch Pharmacopœia
Density 15°/15°C.	0·9180	0·919 to 0·930
Refractive index 20°C.	1·4698	1·466 to 1·471
Solubility in alcohol (80 per cent.)	in ½ to 10 vol.	in 1 vol.
$[\alpha]_D$	−1·3°	—
Cineol (per cent.)	51·5	—
Distillate between 170° and 190°C.	59	66 per cent.

The sample was clear and colourless, or with at most a faint bluish tinge, and the odour was abnormal. Samples recently imported via Macassar also showed abnormal figures, as follows:

	Boeroe	Moluccas	Moluccas
Density 10°/15°C.	1·9148	0·9138	p·9126
Refractive index 20°C.	1·4691	1·4686	1·4674
$[\alpha]_D$	−4·4°	−1·3°	−1·4°
Solubility in alcohol (80 per cent.)	1-10 vols	1·5-10 vols	1-10 vols
Cineol (per cent.)	59·6	60·4	60·4
Fatty Oils	absent	present	trace
Mineral oils	absent	absent	absen

These abnormal figures are apparently due to the difficulties of the reconstruction period in the producing areas. It would appear that the distillation is not carried as far as previously, so that the oil is deficient in heavy components. The presence of small quantities of coconut oil in two samples is also of interest.

G. M.

FIXED OILS, FATS AND WAXES

Fatty Acids, Saturated Straight-Chain, Separation of. L. L. Ramsey and W. I. Patterson. (*J. Ass. Off. agric. Chem.*, 1948, **31**, 441.) The method given depends on partition chromatography and can be used for the straight-chain saturated fatty acids having 11 to 19 carbon atoms. A column of silicic acid is used with a mixture of furfuryl alcohol and 2-aminopyridine as the immobile solvent and *n*-hexane as the mobile solvent. Percolate fractions are titrated with standard sodium ethylate solution, while the separated acids are determined by titration in alcohol (70 per cent.) with standard sodium hydroxide. Each separated acid is tentatively identified by its threshold volume, confirmation being obtained by a melting-point determination or by the addition of an approximately equal amount of an authentic sample of the suspected acid to the unknown, and testing the homogeneity by chromatographic adsorption of the mixture on a fresh column. The separation of the even numbered acids from each other, and of the odd numbered members from each other, was fairly complete in a single fractionation and recoveries of added acids were essentially quantitative. Details of procedure are given, together with results obtained in investigating the purity of a number of fatty acids obtained from commercial sources.

R. E. S.

Neat's Foot Oil, Component Acids and Glycerides of. T. P. Hilditch and R. K. Shrivastava. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 139.) The component acids and glycerides of a sample of Irish neat's foot oil were determined by application of ester fractionation and of low temperature crystallisation from solvents. The acids were fractionated by crystallisation from ether followed by fractionation of their methyl esters. They consisted of myristic 0·7, palmitic 16·9, stearic 2·7, arachidic 0·1, tetradecenoic 1·2, hexadecenoic 9·4, oleic 64·4, octadecadienoic 2·3, octadecatrienoic 0·7, and unsaturated C_{20-22} acids 1·6 per cent. (wt.). The component glycerides, studied after partial separation by low-temperature crystallisation from acetone, were found to include about 35 per cent. of palmitodiolein, 23 per cent. of hexadecenodiolein, 8 per cent. of polyethenoid-diolein, 7 per cent. of oleopalmitostearin and probably not much more than 10 per cent. of triolein, with minor amounts of other mixed glycerides. The presence of fairly substantial proportions of hexadecenoic acid in neat's foot oil had not been previously noted. The specific utility of the oil as a lubricant cannot be connected with a high content of triolein, the present work suggesting the possibility that hexadecenodiolein (nearly one-quarter of the oil) and perhaps also the di-oleoglycerides in which the third acyl group is a polyethenoid member of the C_{18}, C_{20} (or C_{22}) series, may contribute specifically to its lubricant properties. R. E. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Potato Amylose, Action of β-Amylase on. R. H. Hopkins, B. Jelinek and L. E. Harrison. (*Biochem. J.*, 1948, **43**, 32.) The reaction between the purest amylose and β-amylase obtainable has been investigated with special attention to the reaction kinetics. The mixtures used in the investigations and the method for determining the progress of hydrolysis were as described by Hopkins *et al.* (*Biochem. J.*, 1946, **40**, 507) except that in the presence of antiseptics or butyl alcohol the ferricyanide method of Cole was used. It was found that pure potato amylose was hydrolysed by β-amylase, at a steadily declining rate, to completion. Any "denaturation" of the amylose due to undue exposure to water caused a break in the progress curve and incomplete hydrolysis. Amylose crystallised from potato starch paste at *p*H 4, or from autoclaved paste, hydrolysed faster than from non-autoclaved pastes at *p*H 6 or 9. Relatively shorter molecular chains hydrolysed more slowly. Short-chain molecules (less than 12) were not present in amylose prepared by crystallisation methods according to evidence from iodine colours. The blue value of amylose increased with chain length approaching an asymptotic value although it decreased on hydrolysis. The reaction obeyed the formulation of Michaelis and Menten but the value of K_m varied widely with the preparation. A hypothesis is put forward to explain the observed kinetics. R. E. S.

Potato Starch, Fractionation of. R. H. Hopkins and B. Jelinek. (*Biochem. J.*, 1948, **43**, 28.) A number of methods were investigated with the object of obtaining the purest amylose and amylopectin. The processes of Schoch and of Haworth, Peat and Sagrott were used, and amylose and amylopectin were also prepared from non-autoclaved potato starch paste by successive additions on alternate days of cyclohexanol and thymol; the resulting products from the different methods did not show any great differences. Amylose produced under acid conditions showed a greater

tendency to retrograde in neutral solution. The action of hot water on amylose changed even the portion which does not retrograde, the blue value fell, and β-amylose action was incomplete. Preparations of amylose with 17 times the blue value of the corresponding amylopectin were obtained. Purification of amylopectin was less successful, but fractional ethyl alcohol precipitation removed some amylose. R. E. S.

Potato Starch, Fractionation of. Part V. The Phosphorus of Potato Starch. L. H. Lampitt, C. H. F. Fuller and N. Goldenberg. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 121.) Fractions of potato starch, ground for differing periods in a ball mill, were examined for the distribution of phosphorus under various conditions. A small amount only of the phosphorus of potato starch was found to be present in dialysable form. Bound phosphorus was not split off from potato starch fractions by precipitation with alcohol and there were no significant differences between the bound phosphorus in the cold-water soluble and hot-water soluble potato starch fractions. The conclusion was reached that the phosphorus of potato starch is present, either largely or wholly, in the form of strongly-bound esterified phosphate groups, whilst in wheat starch, either all or a large proportion of the phosphorus is present in the form of less strongly-bound, adsorbed phosphatides. The work in the present paper coupled with that of other workers suggests that most of the phosphate groups in potato starch are bound to the amylopectin fraction, whereas in wheat starch the phosphatides are adsorbed, either largely or wholly, by the amylose fraction. The net result of this difference is to increase the hydrophilic character of potato starch solutions and pastes, but to decrease that of wheat starch solutions and pastes. R. E. S.

Potato Starch, Fractionation of. Part VI. Retrogradation of Fractions. L. H. Lampitt, C. H. F. Fuller, N. Goldenberg and G. H. Green. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 179.) The literature concerning the retrogradation (changes in the physico-chemical state that take place in solutions, pastes, or gels on ageing) is briefly summarised and results are reported dealing with the retrograding properties of potato starch fractions as compared with wheat starch fractions. The various cold-water-soluble and hot-water-soluble fractions of starch obtained by grinding potato starch in a ball mill for varying periods were examined and the retrograding properties of the various fractions under varying conditions of temperature and concentration are reported; the properties were qualitatively similar to those of wheat starch fractions published earlier. The differences in retrograding properties of the various fractions depended on the contents of amylose and amylopectin and also on the differences in the nature and distribution of the small quantities of phosphorus present in the two starches. R. E. S.

GUMS AND RESINS

Karaya Mucilage. K. E. Grönkvist. (*Farm. Revy*, 1948, **47**, 623,635.) Karaya gum has been introduced into the new Swedish Pharmacopœia under the name of Gummi sterculiæ. The viscosity of mucilages has been examined, using an Ostwald viscometer. The mucilages were prepared from coarsely powdered gum, and were passed through a homogeniser. When the mucilage was passed repeatedly through the capillary of the viscometer, the apparent viscosity at first decreased, then rose to a constant value. The same pheno-

menon was shown by an alkaline solution, but on ageing the viscosity decreased and became less variable. The maximum viscosity is obtained at *p*H 5 to 7, and decreases sharply on the alkaline side. In all cases of fully hydrated mucilages, the viscosity decreases on keeping, but the decrease is more rapid with a high *p*H value. Heating the dry gum causes the hydration of the mucilage to be very slow, so that such mucilages show a rise in viscosity over a period of some months. The *p*H of the mucilage is not affected by heat, but decreases on storage. G. M.

ORGANIC CHEMISTRY

β-Peltatin, A New Compound from Podophyllin. J. L. Hartwell and W. E. Detty. (*J. Amer. chem. Soc.*, 1948, **70,** 2833.) The fractionation of podophyllin by chromatographic adsorption has yielded 4 per cent. of a new crystalline substance for which the name β-peltatin is proposed. The new compound possesses about the same high necrotising activity for mouse sarcoma 37 as α-peltatin. β-Peltatin crystallises from alcohol in colourless, transparent prisms, m.pt. 231·1 to 238·0°C. (shrinks at 225·5°C.) cor. ; $[\alpha]_D^{20°C.}$ -115° (*c*, 1·009 absolute alcohol). Analysis showed a formula $C_{22}H_{22}O_8$ with three methoxy groups. Molecular weight values (Rast) for derivatives of both α- and β-peltatin agree with the formula $C_{22}H_{22}O_8$ and indicate that the peltatins are thus isomeric with podophyllotoxin. α-Peltatin has one methoxy group less than β-peltatin and podophyllotoxin. Both α- and β-peltatins give an immediate yellow colour with sulphuric acid but with α-peltatin the colour turns reddish-brown, with β-peltatin green, before becoming finally red. R. E. S.

Sodium Citrate, Water of Crystallisation of. T. A. G. Haanappel. (*Pharm. Weekbl.*, 1948, **83,** 687.) The Dutch Pharmacopœia describes crystalline sodium citrate with 5 H_2O. Actually the highest hydrated salt contains $5\frac{1}{2}$ H_2O. The Pharmacopœia also states that the salt effloresces at a relative humidity less than 0·3; investigation showed that this figure should be 0·6. G. M.

Stilbamidine : Instability of. A. J. Henry. (*Brit. J. Pharmacol.*, 1948, **3,** 163.) Hydrolysis of the amidine groups of stilbamidine is a dark reaction. Examination of solution of stilbamidine after storage for three years under various conditions shows that the factors on which the rate of hydrolysis of the amidine groups primarily depends are the *p*H of the solution and the temperature. Storage in the dark at 40°C. of a 1 per cent. solution of the hydrochloride at *p*H 7 will almost certainly produce a good crop of crystals of *trans*-4-amido-4′-amidinostilbene hydrochloride within three months. A *p*H of 5 suppresses hydrolysis almost indefinitely. *Trans*-4-amido-4′-amidinostilbene is more toxic than the parent compound. Its formation in the body—for which conditions of *p*H and temperature would be favourable—from stilbamidine absorbed and retained for long periods may, therefore, in part account for the delayed toxic effects which have been observed. The occurrence of such prolonged storage in the body is supported by recent examination of the urine of kala-azar patients some 18 months after termination of a course of treatment; stilbamidine, or a closely related derivative, is still being excreted at a low level (0·005 to 0·03 mg/100 ml.). It seems highly probable that similar conditions of adsorption, storage and slow release apply for the dimer after administration as appear to apply for stilbamidine. S. L. W.

PLANT ANALYSIS

Alkaloids, Isolation of, From Plants. N. Lörgren. (*Svensk Farm. Tidskr.*, 1949, **53**, 1.) The dried material is first extracted with an organic solvent containing a small quantity of an organic acid which forms insoluble salts with the alkaloids. The alkaloids are then extracted with an organic solvent containing dry ammonia gas. As applied to *Chelidonium majus*, details are as follows. 75 g. of the root is treated with 230 ml. of anhydrous ether containing 2 g. of anhydrous oxalic acid. After shaking for 3 hours, the mixture is filtered and the residue is treated with a further quantity of 150 ml. of ether and 0·5 g. of oxalic acid. After filtering again, the residue is suspended in ether, dry ammonia gas is passed in, and after standing overnight, the extraction is continued in a continuous extraction apparatus for 2 days. The solution is filtered and the ether distilled off. The residue is dissolved in a mixture of 15 ml. of chloroform and 15 ml. of dry ether, then treated with 100 ml. of a saturated solution of anhydrous citric acid in ether. The precipitate is filtered off and dried *in vacuo*. For ergot the defatted drug is extracted exhaustively with ethyl acetate containing ammonia gas. The solution is concentrated at a low temperature, and the alkaloids are precipitated by the addition of a saturated solution of citric acid in ether. The precipitate is allowed to settle, separated by centrifuging, washed with light petroleum, and dried *in vacuo*. It contains all the alkaloids of the drug in very good yield. G. M.

Cascara Sagrada, Chromatographic Isolation of Trihydroxymethylanthraquinones. M. R. Gibson and A. E. Schwarting. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 206.) Chloroformic extracts of extract of cascara sagrada, U.S.P., were run through a 30-mm. tube of celite, 3 parts, and magnesia, 1 part. The red-brown, deep red, orange and pink layers obtained were separated, treated with 10 per cent. hydrochloric acid to dissolve the magnesia and shaken out with chloroform. When the chloroformic solutions were run through 12 mm. tubes of the same adsorbent, the red-brown layer proved to be a mixture of the anthraquinones, whereas the deep red, orange and pink layers were homogeneous, and contained the three anthraquinones (emodin, aloe-emodin and *iso*emodin) each contaminated with an unknown substance, which could be removed by subliming under reduced pressure. The progress of the separation and purification was followed by similar experiments on various mixtures of the pure anthraquinones, and by spectrophotometric measurements. The method is not suitable for quantitative isolation of the anthraquinones unless the nature of impurities appearing in the first resins is known. A pale yellow layer which appeared immediately below the adsorbed anthraquinones in all experiments was not identified but was shown not to interfere appreciably with the spectrophotometric measurements over the range of wave-lengths used. The use of spectrophotometry showed its suitability as a method for the quantitative analysis of mixtures of the anthraquinones. The method described for the preparation of pure *iso*emodin was a modification of that of Green, King and Beal (*J. Amer. pharm. Ass.*, 1938, **27**, 95) and gave bright orange crystals melting at 179°C. G. R. K.

Pyrethrin Content of *Chrysanthemum cinariæfolium* flowers. M. G. Edwards. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 379.) The pyrethrins were rapidly extracted from the undried flowers by grinding 400 g. with 120 g. of light petroleum (55° to 60°C.) in a rod mill for 2 hours. A further 500

g. of light petroleum was added, grinding continued for 2 minutes and the marc washed until the washings were colourless. In this way some 95 per cent. of the pyrethrins were extracted: the remainder can be obtained by a 7-days' maceration or in a Soxhlet. Three strains of flowers were investigated—(a) large flowers of low pyrethrin content; (b) "high toxic" flowers; (c) commercial Grade 1 flowers. To obtain a comparison with moisture-free flowers, samples were dried (a) in a draught at room temperature, for 12 to 14 days; (b) in a current of air at 55°C., for 7 to 8 hours; (c) in a commercial drying tunnel at 55°C., for 24 hours. The main extract was concentrated and the pyrethrins determined in aliquot portions by the A.O.A.C. method, modified by the use of hydrochloric acid in place of sulphuric acid to acidify before the light petroleum extraction of the monocarboxylic acid. The free chrysanthemum acids were not extracted and are included in the pyrethrins. Tables show the pyrethrins in the extracts of fresh flowers, and corresponding dried flowers, calculated to moisture-free basis. The results indicate that the undried flowers contain about the same amount of pyrethrin I, and about 10 per cent. more of pyrethrin II than the same flowers dried in the most favourable way, and 3 to 4 per cent. more pyrethrin I and 12 to 13 per cent. more pyrethrin II than flowers dried by one particular commercial method. H. F.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Antipyretic Action and Catalase Activity. J. Williamson and E. A. Rudge. (*Biochem. J.*, 1948, **43**, 15.) Many antipyretics can be used to stabilise hydrogen peroxide solution and several representative antipyretic substances were therefore tested for inhibitory activity on the isolated catalase-hydrogen peroxide system in an attempt to provide evidence for the hypothetical suggestion that such substances lower the temperature of the body by reducing the rate of oxidation, conceivably by interfering with hydrogen peroxide metabolism. Of the substances tested, acetanilide and phenacetin represented typical aniline derivatives, salicylic acid and acetylsalicylic acid represented typical phenol derivatives, phenazone represented pyrazolone derivatives, and quinine represented quinoline derivatives; *p*-hydroxybenzoic acid was also examined. Parallelism with antipyretic activity was not found since inhibition of the *in vitro* catalase-hydrogen peroxide system was observed with some, but not all, of the substances tested. A horse liver catalase extract was used; phenacetin, phenazone and quinine showed no inhibition; acetanilide showed a slight inhibition, the greatest inhibitory action being obtained with salicylic acid, acetylsalicylic acid and *p*-hydroxybenzoic acid. It is suggested that the observed inhibitions with the hydroxybenzoic acid derivatives result from the presence of phenolic -OH groupings. R. E. S.

Bacitracin. Production and Properties of Crude Substance. H. S. Anker, B. A. Johnson, J. Goldberg and F. L. Meleney. (*J. Bact.*, 1948, **55**, 249.) Bacitracin, which was first extracted from a culture of an organism of the *Bacillus subtilis* group isolated from an infected wound, has not yet been obtained in the pure state. An arbitrary unit has been defined as the amount which when diluted 1 in 1024 completely inhibits the growth of a stock strain of group A hæmolytic streptococcus. It can be assayed by a serial dilution method, or by a plate method using

strains of *Corynebacterium*. Suitable media for production purposes are an *l*-glutamic acid synthetic medium or a soya bean digest medium. Extraction cannot be effected by a number of common solvents but *n*-butanol extracts 85 to 90 per cent. of the activity. The butanol is distilled from the extract in the presence of water, giving a 100-fold concentration. Purification is effected by butanol-ether extraction at *p*H 3 to 4, the activity remaining in the aqueous layer. The latter is extracted with ether, distilled under reduced pressure, and freeze-dried after neutralisation. An alternative method of purification using magnesium oxide with subsequent precipitation as the salicylate is also described. Aqueous solutions of crude bacitracin are stable at 0° to 5°C. for 8 to 12 months, but at higher temperatures there is a loss of activity which is complete at 37°C. in two weeks. Alkali causes rapid inactivation; so also does hydrogen peroxide, but thiol compounds cause no measurable change. Thioglycollic acid partially reactivates peroxide-inactivated material. The substance is soluble in alcohols but insoluble in many other organic solvents. Diffusion experiments indicate a molecular weight below 2000. It is precipitated by heavy metals and by several organic acids, sometimes, for example, by salicylic acid, without loss of activity. Chemical examination shows that the substance is not a peptide, and that it lacks guanido and phenolic groups. H. T. B.

Diphtheria Toxoid: Improvement in Preparation. L. B. Holt. (*Brit. J. Exp. Path.*, 1948, **29**, 343.) Diphtheria toxoid produced in the ordinary way is first treated with magnesium hydroxide to remove colour, inorganic phosphates, and some protein. It is then treated with cadmium chloride solution and finally fractionated with ammonium sulphate. H. T. B.

Liver Extract, Purified; Chemical Nature as Determined by Paper Partition Chromatography. G. H. Tishkoff, A. Zaffaroni and H. Tesluk. (*J. biol. Chem.*, 1948, **175**, 857.) Results are given for the investigation of a commercial, highly purified, liver extract containing the antipernicious anæmia factor using two-dimensional paper partition chromatography. The presence of one or more polypeptides of high molecular weight was proved; the polypeptide material was separated and the free amino acids were liberated from it by hydrolysis. The following amino-acids were obtained: leucine, glycine, alanine, aspartic acid, valine, proline (relatively large amounts); arginine, lysine, glutamic acid; serine, phenylalanine, threonine, hydroxyproline (medium amounts); histidine, cysteine, methionine (small amounts). Some free amino acids were found in the original preparation and also riboflavine, but folic acid and xanthopterin were not found. R. E. S.

Penicillin, Radioactive, Studies with. D. Rowley, J. Miller, S. Rowlands and E. Lester Smith. (*Nature*, 1948, **161**, 1009.) In view of the possibility that penicillin may act by depriving sensitive organisms of glutamic acid, which is probably essential to their growth, the authors have attempted to ascertain whether penicillin, by being itself absorbed, blocks the passage of glutamic acid through the cell wall. As the amount of penicillin taken up by organisms is too small to detect by biological methods, it was decided to attempt its detection by using radioactive penicillin of a high specific activity, namely, 0·05 microcurie per I.U., the radioactivity of the penicillin being measured by means of a Geiger-Müller counter having a background of 14 counts per minute. For the absorption experiments, *Staphylococcus aureus* was grown on agar plates

at 37°C. for 9 hours, scraped off, transferred to 25 ml. of broth, so as to give a thick suspension, and incubated for a further 2 hours. A measured amount of radioactive penicillin was then added and an aliquot portion of the suspension removed for radioactive assay, this removal of a standard volume of the suspension being repeated at intervals as a control. After ½, 2, 6 and 24 hours, 5 ml. of the suspension was removed and filtered through a "Gradocol" membrane, and washed through with 2 ml. of water into a graduated receiver. Aliquot portions of these filtrates were taken, dried, weighed and counted. Any uptake of penicillin by the bacteria should be shown by a decrease in penicillin concentration in the filtrate. From the results of an experiment in which 0·07 I.U. per ml. was employed in a suspension containing $3{\cdot}2 \times 10^9$ viable organisms per ml., it was seen that the greatest difference in the penicillin concentrations in the filtrates was no greater than that for the suspensions. Corresponding results were obtained from other experiments, using penicillin concentrations of from 1 down to 0·05 I.U./ml. From these experiments the authors conclude that absorption of penicillin, if any, probably amounts to less than 10 molecules per bacterium. S. L. W.

Pyridine Nucleotides, Extinction Coefficients of the Reduced Band of. B. L. Horecker and A. Kornberg. (*J. biol. Chem.*, 1948, **175,** 385.) Precise values for the extinction coefficients of diphosphopyridine nucleotide and triphosphopyridine nucleotide were determined on partly pure preparations by the use of pure substrates in reactions which are essentially complete. Such determinations have been made with pyruvic acid, acetaldehyde and *iso*citric acid. The systems actually used were pyruvate di- and tri-phosphopyridine nucleotide, *iso*citrate-triphosphopyridine nucleotide, and acetaldehyde-diphosphopyridine nucleotide. A molecular extinction coefficient of $6{\cdot}22 \times 10^6$ for the reduced forms of both di- and tri-phosphopyridine nucleotides at 340mμ. was obtained. R. E. S.

BIOCHEMICAL ANALYSIS

Ascorbic Acid in Food Preparations, Estimation of. S. A. Goldblith and R. S. Harris. (*Anal. Chem.*, 1948, **20,** 649.) It is shown that both the indophenol method and the method based on coupling with 2 : 4-dinitrophenylhydrazine give satisfactory results in estimating the ascorbic acid content of fresh vegetables. The indophenol technique measures biologically active ascorbic acid while the dinitrophenylhydrazine method measures *l*-ascorbic acid, dihydroascorbic acid and 2 : 3-diketogulonic acid. The ascorbic acid in oxalated slurries (with four parts of 0·5 per cent. oxalic acid) was oxidised almost completely to dehydroascorbic acid in 21 days while the values obtained by the dinitrophenylhydrazine method remained constant for 14 days. It is claimed that inasmuch as the indophenol and dinitrophenylhydrazine methods agree when used to measure ascorbic acid in ground-fresh plant materials and disagree more and more during storage after the plant is taken from the ground, these methods should prove useful in checking the freshness of perishable vegetables. The ascorbic acid content of garden-fresh edible plants may thus be measured in a laboratory remote from the harvest area. Both methods may be employed to establish the freshness of vegetable foods. R. E. S.

Barbiturates in Blood and Tissues, An Ultraviolet Spectrophotometric Procedure for the Determination of. L. R. Goldbaum. (*J. Pharmacol.*, 1948, **94,** 68.) A simple, rapid and highly specific procedure is described

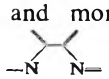
for the determination of barbiturates in blood and tissues based on the characteristic ultraviolet absorption spectra of the malonylurea ring structure. The drug is extracted by an organic solvent, usually chloroform, and then re-extracted with alkali. The ultraviolet absorption spectra of the alkaline solution is determined against a reference blank solution of sodium hydroxide using the photoelectric quartz spectrophotometer. Absorption spectra of some representative barbiturates, e.g., seconal and amytal, showed an intense ultraviolet absorption with a maximum at 225mμ and a minimum of 235mμ. At 255 mμ the concentration bears a linear relationship to the optical density up to at least 20 μg./ml. for these barbiturates. The method is sensitive to 0·4 mg./100 ml. of blood and 1·0 mg./100 g. of tissue, with an error of less than 10 per cent. E. N. I.

Heparin, Assay of. C. N. Mangieri. (*J. Lab. clin. Med.*, 1947, **32**, 901.) Fresh bovine blood containing 50 ml. of 8 per cent. solution of sodium citrate per l. is used. The plasma may be used fresh or after storage at −20°C. with equal accuracy. It is recalcified before assay with a predetermined amount of calcium chloride dissolved in 0·2 ml. of physiological saline solution so that the end-point for clotting lies near the middle of the series of twelve tubes. Two series of tubes are set up, one containing the standard and the other, the unknown heparin. 10 μg./ml. of standard or purified heparin or 20 to 40 μg. of crude heparin is used. The standard is diluted to 1·0 Toronto unit/ml. The first tube of each series contains 0·23 ml. of heparin solution and this is increased by 0·02 ml. up the series. The volume of every tube is then made up to 0·8 ml. with saline solution. To the first tube of each series 1·0 ml. of plasma and 0·2 ml. of the calcium chloride solution are added. The contents are gently mixed and incubated at 37°C. for 3 hours. This is repeated with each 2 corresponding tubes in the series at a time. The end-point is the tube in which clotting is just prevented after 3 hours. Fluorescent light is recommended for detecting thin films of clot. The activity of the heparin under test is obtained from the quantities of heparin in each of the end-point tubes. The author claims that the end-point is definite and that results are reproducible with less than 10 per cent. of error. A. D. O.

Theophylline in Blood and Urine, Determination of. A. J. Plummer. (*J. Pharmacol.*, 1948, **93**, 142.) To 4 ml. of a methyl alcohol solution of theophylline in a 15-ml. centrifuge tube add 5 ml. of a saturated solution of copper acetate, and allow to stand tightly-stoppered for 4 hours to ensure complete precipitation of the theophylline-copper compound; centrifuge for 15 minutes at 1000 r.p.m.; decant the supernatant fluid, drain, and wash the precipitate with 5 ml. of methyl alcohol; again centrifuge, decant, and drain; dissolve the precipitate in 4 ml. of 0·2N sulphuric acid and add 0·5 ml. of potassium iodide solution (1 g. to 1 ml. of water); titrate the liberated iodine with 0·02N sodium thiosulphate, using soluble starch solution as indicator; each mg. of theophylline is equivalent to 0·57 ml. of 0·02N sodium thiosulphate. For blood, deproteinise by adding 25 parts of blood to 40 parts of 13 per cent. trichloracetic acid; allow to stand 20 minutes; filter or centrifuge; render just alkaline to litmus with 2·5N sodium hydroxide; add 10 ml. of a phosphate buffer of *p*H 8·0, the final *p*H must be between 7·3 and 8·2; extract the theophylline by shaking the buffered filtrate with three 20-ml. quantities of a mixture of 3 volumes of chloroform and 1 volume of isopropyl alcohol for 5 minutes each extraction; evaporate the combined extracts just to dryness on a water-bath;

dissolve the residue in methyl alcohol; transfer the solution to a 15-ml. graduated centrifuge tube, keeping the final volume of methyl alcohol between 0·3 and 0·5 ml.; determine as previously described, using 0·005N sodium thiosulphate solution for the final titration; each mg. of theophylline is equivalent to 2·28 ml. of 0·005N sodium thiosulphate. For urine, adjust the *p*H of the urine to between 7·3 and 8·2, and continue with the determination as for blood, commencing with the words "extract the theophylline . . ."; the volume of methyl alcohol used to dissolve the theophylline should be from 1 to 2 ml., and 0·01N sodium thiosulphate should be used for the final titration; each mg. of theophylline is equivalent to 1·14 ml. of 0·01N sodium thiosulphate. The method is sensitive to 0·13 mg. of anhydrous theophylline per 100 ml. of blood or urine. From 15 to 20 ml. samples of blood are satisfactory. A dilute urine may be used directly, but concentrated urine should be diluted with 3 or 4 volumes of water. Water should be rigidly excluded from the solutions when precipitating the theophylline with copper acetate, or the precipitation may not be quantitative. Caffeine, theobromine, uric acid, ethylenediamine and sodium acetate do not interfere with the determination, nor does any normal blood property or constituent. S. L. W.

CHEMOTHERAPY

Anthelmintic Potency in Relation to Chemical Constitution. E. Baldwin. (*Brit. J. Pharmacol.*, 1948, **3**, 91.) A report on the results of tests carried out *in vitro* on over 200 chemical compounds for the detection of anthelmintic potency, using an *Ascaris* preparation of which the muscle is directly exposed to the action of the drug. Significant activity was found among aliphatic-aromatic and aromatic-aromatic ketones, but nothing approaching the activity of santonin was discovered in this group. Considerable activity was observed among lactones, but here again none approached the activity of santonin. These facts seem to support the suggestion that the outstanding anthelmintic efficacy of santonin is due to the simultaneous presence of both ketonic and lactonic groups in its structure, rather than to either alone. Among the lactones, phenols and pyridines tested it was noted that anthelmintic activity increased with the addition of a second (usually a benzene) ring to the parent molecule and that activity was greater when the two rings were independent than when they were fused. The value of phenolic carbamates was confirmed, and an unusually high order of potency demonstrated in 2-hydroxydiphenyl carbamate. None of the thiazoles examined showed much promise of useful potency, but among the pyridines an outstanding order of activity was shown by 4-benzylpyridine, and more especially by 2-2′-dipyridyl and 4:5-phenanthroline. The  linkage in the last two compounds and in the corresponding tripyridyl possesses properties which seem to offer considerable possibilities in the search for new and highly efficacious anthelmintics. No activity was discovered among a number of microbial antibiotics, and there is no reason to think that penicillin, or the sulphonamides can yield new anthelmintic agents of any practical value. The importance of using experimental material of nematode origin as the basis of methods of this kind is strongly emphasised. S. L. W.

Antimalarial Compounds. Studies in the Chemotherapy of Tuberculosis. E. Hoggarth and A. R. Martin. (*Brit. J. Pharmacol.*, 1948, **3**, 156.)

Antituberculous activity in mice has been demonstrated with a new group of compounds, 2-arylamino-4-dialkylamino-6-methyl-pyrimidines, some members of which are active as antimalarial drugs. The compound showing most promise was 2-*p*-chloroanilino-4-δ-diethylamino-α-methylbutylamino-6-methylpyrimidine dihydrochloride (No. 3300). No activity was found with the other antimalarial drugs tested, including quinine, mepacrine and pamaquin. S. L. W.

Diaminomethylpyrimidines and Related Compounds: Studies in the Chemotherapy of Tuberculosis. E. Hoggarth, A. R. Martin, M. F. C. Paige, M. Scott and E. Young. (*Brit. J. Pharmacol.*, 1948, **3**, 160.) More than 100 diaminomethylpyrimidines and related compounds have been examined for antituberculous activity in mice. The aim of the investigation was to discover a compound with greater activity than that possessed by No. 3300 (*Brit. J. Pharmacol.*, 1948, **3**, 156). This aim was not realised, and it would appear that in compound No. 3300 itself and a number of closely related compounds the maximum activity possible in this particular chemical group has been reached. S. L. W.

Sulphonamides: Studies in the Chemotherapy of Tuberculosis. E. Hoggarth, A. R. Martin and E. H. P. Young. (*Brit. J. Pharmacol.*, 1948, **3**, 153.) The activity of a series of 2-sulphanilamido-4 : 6-dialkoxypyrimidines and some closely related compounds against *M. tuberculosis in vitro* has been studied. In the former group, activity *in vitro* increases with increasing size of the alkoxy groups, but activity is limited by the consideration that substituents sufficiently large to confer high activity *in vitro* result in such poor adsorption that activity *in vivo* cannot be expected. The di-*n*-propoxy and di-*iso*propoxy compounds produced a significant increase in the mean survival times of groups of mice infected with *M. tuberculosis* when the drug was given both before and after the mice were infected, but when drug treatment was delayed for 24 hours no therapeutic effect was demonstrated. The higher members of the series were very poorly absorbed and failed to show any therapeutic action. S. L. W.

PHARMACY

DISPENSING

Sodium Citrate, Sodium Chloride and Glucose, Preparation of Sterile Solutions of. P. G. Hoorweg and G. V. D. Reyden. (*Pharm. Weekbl.*, 1948, **83**, 684.) A solution containing 3·3 per cent. of sodium citrate, 5 per cent. of glucose, and 0·9 per cent. of sodium chloride was found to develop a turbidity on storing, especially under tropical conditions. The deposit consisted of silicates, although a specially hard glass was used for the containers. The precautions which were found necessary to prevent this precipitation were found to be the following: use of special hard glass; closures of a suitable plastic, and not of rubber; washing the asbestos filter, used to remove pyrogen, with a very large quantity of distilled water before use. G. M.

Sulphathiazole for Injection, Sterilisation of. P. Morch. (*Arch. Pharm. Chemi*, 1948, **55**, 575.) Sulphathiazole dissolves in alkali with a yellow colour, which is increased on heating. In alkaline solutions the compound is actually present as a resonance form and hydrolysis may occur. The author

describes a method for determining 3 of the products as a measure of the degree of decomposition. Results are summarised thus:

Atmosphere in ampoule	Heat treatment	Decomposed per cent.
Air	1 hour at 100°C.	0·04
Air	20 minutes at 120°C.	0·08
Air	1 hour at 120°C.	0·28
Nitrogen	20 minutes at 120°C.	0·05
Oxygen	20 minutes at 120°C.	0·12

The discoloration of the solutions was proportional to the amount of decomposition. The preparation is very sensitive to light: after standing in sunlight for three weeks the solution became dark red and had decomposed to the extent of about 3 per cent. The content of sulphathiazole was not appreciably changed by heat treatment. It is concluded that sterilisation of a sulphathiazole solution (*p*H about 9·5) should be carried out at 120°C. for 20 minutes. G. M.

GALENICAL PHARMACY

Morphine and Apomorphine, Stability of Solutions of. A. Ionescu-Matiu, A. Popescu and L. Monciu. (*Ann. pharm. Fr.*, 1948, **6**, 137.) The degree of decomposition of a solution of morphine or apomorphine may be determined by determining the ferricyanide value, as follows. To 1 to 5 ml. of a 1 per cent. solution of morphine 4 ml. of ferricyanide reagent (4 per cent. of potassium ferricyanide with 4 per cent. of potassium hydroxide), and 20 ml. of water are added. After boiling for 5 minutes and cooling, 50 ml. of water is added, followed by 5 ml. of sulphuric acid (20 per cent.). The mixture is then titrated with permanganate until a pink colour persists for 1 minute. By using this reaction, it was shown that a solution for injection of morphine hydrochloride had undergone from 2 to 12 per cent. of decomposition in 1 year, according to the conditions under which it was kept. The decomposition may be prevented by the addition of sulphite according to the following formula: morphine hydrochloride 0·40 g., sodium bisulphite solution (10 per cent.) 1 drop, water to 10 ml. Sterilisation is for 20 minutes at 100°C. A corresponding formula is used for apomorphine. In tinctures and other liquid galenical preparations, the ferricyanide process may be applied to the extracted morphine. The results showed that the degree of decomposition in one year did not exceed 15 per cent. It is recommended that these preparations should be stabilised with sulphite or benzoic acid. G. M.

PHARMACOGNOSY

***Atropa Belladonna*, Frequency Determinations of.** D. D. Boswijk. (*Pharm. Weekbl.*, 1948, **83**, 609.) Frequency determinations of *Atropa Belladonna* have now been extended to 6 further samples from different localities. The results in general agree with those found previously (*Pharm. Weekbl.*, 1948, **83**, 225; *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 534), but a sample from Leyden showed an abnormal frequency for the stomata in the upper epidermis, ranging from 63 to 88. In samples from Groningen, Delft and Leyden, trichomes with unicellular stalks and multicellular glands were prominent; while multicellular stalks and unicellular glands were general in *Atropa Belladonna* var. *lutea*. G. M.

***Cinchona Ledgeriana* Bark. Distribution and Interrelationships of Alkaloids in.** H. F. Birch and L. R. Doughty. (*Biochem. J.*, 1948, **43**, 38.) The distribution of alkaloids throughout the whole bark of 3 trees of *Cinchona Ledgeriana*, each 7 years old, was investigated. Two of the trees had suffered damage at an earlier stage of their growth and differed markedly in shape from the third tree which was of apparently normal growth and straight in form. Samples of bark were analysed for total alkaloids, quinine, cinchonidine, and for amorphous alkaloids. The distribution of alkaloids in the stem bark was a function of bark thickness which is at any point inversely proportional to the distance of that point from the base of the tree. This basic regular decrease in alkaloid content from the base of the tree upwards was interrupted by zones of relatively high alkaloid content due to local thickness of the bark where the main stem forked, usually caused by damage of the stem at an earlier stage in the tree's life, where secondary leaders arose and where large branches joined the stem. Significant increases in alkaloidal content associated with unit increase in bark weight were found for total alkaloids, quinine, and cinchonidine/unit increase in bark weight, and for quinine, cinchonidine, and cinchonine/unit increase in total alkaloids. These increases were large compared with the small and insignificant increases (and decreases in the case of one tree) found for the amorphous alkaloids, an indication of the progressive conversion of amorphous to crystallisable alkaloids during the life of the tree. When the trees were felled the amorphous alkaloid content was that currently available for transformation to the crystallisable alkaloids, while the crystallisable alkaloid content represented the accumulation of the end products of the amorphous alkaloid transformations throughout tree growth. Cambial activity further governed the efficiency or degree of conversion of the amorphous to the crystallisable alkaloids, for in one tree the highest crystallisable to amorphous alkaloid ratios obtained where the tree bent and where the secondary leader arose. In general, the distribution of the alkaloids throughout the bark is governed primarily by the history of the tree as reflected in its form. R. E. S.

***Datura Stramonium*, Growth Effects produced by 2 : 4-Dichlorophenoxyacetic Acid applied to the Stems.** H. W. Youngken, Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 196.) Hydrous wool fat containing 2:4-dichlorophenoxyacetic acid in concentrations varying from 0·0001 to 5 per cent. was applied to the stems of seedlings about 3 in. high and older plants about 8 to 10 in. high. Seedlings treated with 0·0001 and 0·001 per cent. showed no abnormal effects, but higher concentrations produced a systemic effect proportional in degree to the concentration used. The effect was manifested by stunted growth, skin bending and swelling, cellular proliferation and roughening and curling of the leaves. Seedlings did not recover from the effects if the concentration was greater than 1 per cent. Those older plants treated with 1 to 5 per cent. showed, after 45 days, stunted growth, swollen stem bases with warty outgrowths, marked curvature of the stems above the first branching, folding of the leaves, increased pubesence along the leaf veins and petioles, and retardation of flower development, only the lower flowers maturing; other flower buds which formed remained unopened. Two plants treated with 1 to 0·1 per cent. and on which mature fruits formed produced spineless capsules. After three weeks the seeds of these fruits were chocolate brown and either flattened and kidney-shaped, or vermiform to crescent or horn-shaped, and smaller. Older plants treated with 0·1 per cent. or less showed little if any toxic effects. The determination of the total alkaloids of leaves from plants which were 85 days old and had received four

applications of 0·001 per cent. of 2:4-dichlorophenoxyacetic acid showed no significant difference from the control plants. Since, however, stronger concentrations produce somewhat drastic effects on the leaves, the effect of these on alkaloidal formation is being investigated. G. R K.

PHARMACOLOGY AND THERAPEUTICS

Alcohol, Sensitisation to, by Drug. J. Hald and E. Jacobsen. (*Lancet*, 1948, **255**, 1001.) Doses of 0·5 to 1·5 g. of diethylthiuramdisulphide [bis(diethylthiocarbanyldisulphide)], $(C_2H_5)_2NC(S).S.S.C(S)N(C_2H_5)_2$ although without effect by themselves produce unpleasant symptoms if alcohol is taken subsequently. The symptoms following 10 to 20 g. of alcohol include flushing of the face, dilatation of the scleral vessels, palpitations and possibly slight dyspnœa. Larger doses of alcohol cause nausea and vomiting. This sensitisation seems to be due to a great increase in blood acetaldehyde which occurs when both the drug and alcohol are taken, but which is absent when either is taken separately. Sensitisation begins about 3 hours after the drug is taken and may last about 48 hours depending on the dose. The drug is stated to be non-toxic by itself and is excreted very slowly. W. W. W.

Alcoholism treated by Sensitising Drug. O. Martensen-Larsen. (*Lancet*, 1948, **255**, 1004.) The author has used tetraethylthiuramdisulphide in the treatment of 83 cases of alcoholism. This drug although innocuous by itself causes unpleasant symptoms if alcohol is taken subsequently. 74 of the patients developed distaste for alcoholic drinks as a result of the treatment; 9 refused to continue with it. After physical and psychiatric examination the patient received 1·0 to 1·5 g. of the drug followed by 0·5 g. daily, and informed of the consequences should he drink alcohol, but he is encouraged to try it to show the effect of the treatment. Although heavy drinkers can still take a fair amount of alcohol at the start of the treatment, their tolerance for alcohol soon diminishes and finally all desire for drink seems to be lost. W. W. W.

Analgesics, A Method of Testing in Man. A. J. H. Hewer and C. A. Keele. (*Lancet*, 1948, **255**, 683.) Instead of the usual method of testing analgesics by measuring the threshold intensity of some stimulus required to elicit pain, tests have been made on the power to relieve pain experimentally induced by the contraction of ischæmic muscles. By means of a cuff the distal circulation of the arm was cut off and the hand and forearm muscles were then exercised by rhythmically compressing a bulb. After 50 to 60 contractions had been made there was slight or moderate pain in the forearm muscles. The contractions were then stopped and the ischæmia still maintained. Soon the pain began to increase and became intolerable after 8 to 15 minutes. If the cuff was deflated the pain disappeared in a few seconds. Ten grades of pain could be distinguished and are described in units from 1 to 10. The drugs were given when the pain was of the value of 4 to 5 units, gases by inhalation, the others by intravenous injection, thus eliminating differences due to different rates of absorption. It was found preferable, instead of trying to find the smallest dose that would give complete relief from pain of 4 to 5 units of intensity, to find the smallest dose that would produce a definite effect at this stage. For this purpose a narrowing range of doses, well above and below the threshold, were given, until there was only a small differ-

ence between the effective and ineffective doses. Nitrous oxide from 10 per cent. to 40 per cent. in oxygen, and cyclopropane, 2 and 3 per cent. in oxygen, were used. Results are given for the following drugs: (1) morphine (hydrochloride or sulphate); (2) pethidine hydrochloride; (3) *dl*-2-dimethylamino-4:4-diphenyl-heptan-5-one hydrochloride, amidone; (4) *dl*-1-dimethylamino-3:3-diphenyl-hexan-4-one hydrochloride (Hoechst 10582); (5) *dl*-2-dimethylaminomethyl-3:3 diphenyl-hexan-4-one hydrochloride (isoamidone); (6) *dl*-2:1-morpholino-4:4 diphenyl-heptan-5-one hydrochloride (C.B. 11); (7) procaine hydrochloride; (8) thiopentone sodium; (9) benadryl hydrochloride; (10) N-*p*-methoxybenzyl-N-dimethylaminoethyl-2-aminopyridine maleate (neoantergan, antistin, pyranisamine maleate); (11) N-phenyl-N-benzyl-2-methylimidoazoline (antisan); (12) tetraethylammonium bromide. The results show great variations between the 4 persons studied and clinical differences in response to these drugs can, in part, be ascribed to differences in sensitivity towards them. It was found, for example, that amidone and C.B.11 were both 30 times as potent as pethidine, while in another subject morphine and amidone were both 10 times as potent as pethidine but only 1/10 as powerful as C.B.11. Again, in another subject morphine, amidone and C.B.11 were about equally analgesic and were 20 to 25 times more potent than pethidine. H. F.

Calciferol by Intramuscular Injection. T. Lightbound. (*Lancet*, 1948, **255**, 1010.) Initially, patients with lupus vulgaris, lupus verrucosus, erythema induratum and normal controls were treated orally with 150,000 I.U. daily of calciferol for 6 months. The dose had to be reduced in some patients owing to nausea and vomiting. To overcome this, intramuscular injections of 600,000 I.U. were given thrice weekly for 3 weeks and then twice weekly. The intramuscular route is preferred because results were more rapid, pigmentation was absent, there were no toxic symptoms, there was little or no hypercalcæmia, less chance of reduced packed-cell volume and of raised blood urea. All cases, however, showed diminished kidney function (urea clearance test), and therefore treatment should not exceed 4 months without a rest period. W. W. W.

Chloroquine, Chronic Oral Toxicity of. O. G. Fitzhugh, A. A. Nelson and O. L. Holland. (*J. Pharmacol.*, 1948, **93**, 147.) A 2-year chronic toxicity study with rats fed on diets containing from 100 to 1,000 p.p.m. of chloroquine showed that the toxicity of the drug was very slight or questionable at 100 p.p.m. and became progressively more severe with each increase in dosage. There was a significant retardation of growth at a concentration of 400 p.p.m. A progressive increase in mortality occurred at dosage levels of 200 p.p.m. or more, and 800 and 1,000 p.p.m. caused early death of all animals. The outstanding hæmatological change was a leucocytosis, predominantly neutrophilic, marked in the group on 800 p.p.m., less striking in the group on 400 p.p.m., and scarcely noticeable in the group on 200 p.p.m.: there was increase in the hæmoglobin concentration and erythrocyte counts in the rats on 800 p.p.m. Histopathological changes increased from very slight or absent in rats on 100 p.p.m., to marked in those on 800 and 1,000 p.p.m.: the two prominent lesions at toxic doses were a slow focal necrosis of striated muscle, especially cardiac, and a moderate degree of centrolobular hepatic necrosis and fibrosis. In relation to bodyweight of the rat, the lowest dosage of chloroquine which produced slight toxic effects in some animals corresponds to approximately 4 mg./kg./day for 2 years. This corresponds

approximately to the prophylactic dosage in man; however, taking the length of time into consideration, the amount of chloroquine that will produce toxic effects in rats is above the therapeutic or prophylactic dose for man. The toxicity of chloroquine was found to be slightly less than that of mepacrine; at the low dosage level of 4 mg./kg./day there was no noticeable difference between the toxicities of the two substances. S. L. W.

Dimercaprol in the Treatment of Experimental Lead Poisoning in Rabbits. F. G. Germuth and H. Eagle. (*J. Pharmacol.* 1948, **92,** 397.) Rabbits which had received 5 consecutive daily subcutaneous injections of lead acetate in a dosage of 240 mg./kg. all died in from 3 to 40 days, the survival time averaging 26 days after the last injection. A series of animals receiving this dosage of lead acetate were then treated for 5 days with dosages of dimercaprol varying from 20 to 80 mg./kg. daily. Dimercaprol significantly hastened their death, the average survival time at the smallest dosage of dimercaprol being reduced from 26 days to 15 days, and at the highest dosage from 26 days to from 1 to 12 days, the mortality being greatest within the first four days. This experiment was carried out in the hottest part of the summer; in a second similar experiment carried out in the winter months dimercaprol did not accelerate death but it still failed to exert a protective action. Similar results were found in acute lead poisoning induced by intravenous injections of lead acetate, the animals treated with dimercaprol dying approximately in the same time as the untreated controls. Further experiments to determine the effect of dimercaprol on urinary lead excretion showed that it caused a striking increase. For two hours after a single injection of dimercaprol at 20 mg./kg. the urinary excretion of lead increased 11- to 40-fold in animals with a subcutaneous depot and 3- to 7-fold in animals injected intravenously, this favourable effect lasting for 4 hours after a single injection. The magnitude of the lead-excretion response decreased with each additional injection of dimercaprol, suggesting that only a small proportion of the lead injected could be dissociated by dimercaprol from its combination with the tissues. The reason for the failure of dimercaprol to protect the animals in spite of increased urinary lead excretion may be due in part to the fact that only a small portion of the total body store of lead is mobilised and the amount eliminated is too small to effect the outcome. A second reason for failure may be the fact that the lead mobilised by dimercaprol is shown to form a lead-dimercaprol complex which is almost as toxic as lead acetate itself, which may explain why in some experiments the administration of dimercaprol actually accelerated death, since the lead complex when formed may act on other organs more vulnerable to the toxic effects of lead, or more vital to the host, than were the tissues in which the lead was originally deposited. In spite of these results, the authors consider, in view of the striking effect of dimercaprol on the urinary excretion of lead, its cautious therapeutic trial in man is justified. S. L. W.

Hetrazan, Mode of Action in Filariasis. F. Hawking, P. Sewell and P. Thurston. (*Lancet,* 1948, **255,** 730.) Experiments were carried out on cotton-rats infected with *Litomosoides carinii,* to study the mode of action of a new compound, hetrazan (1-diethylcarbamyl-4-methylpiperazine), which has been introduced for the treatment of human filariasis due to *Wütchereria bancrofti.* Intravenous injection of 6 mg./100 g. was followed by rapid diminution of the microfilariæ, 80 per cent. disappearing in 1 minute and over 90 per cent. in 2 minutes. Microfilariæ surrounding

the adult worms in the pleural cavity remain active despite intensive treatment and form a reservoir from which the supply in the blood is constantly replenished. Hetrazan has little effect on the adult worms. Experiments *in vitro*, and the histological examination of the distribution of the microfilariæ in the different organs suggests that hetrazan has an opsonin-like action on the microfilariæ and renders them susceptible to destruction by the reticuloendothetial system. Microfilariæ *in vitro*, and in the pleural cavity are not in contact with phagocytes and are not so rapidly affected by hetrazan. E. N. I.

Niaara: a Digitalis-like Colombian Arrow-Poison. K. Mezey, C. Uribe-Piedrahita, J. Pataki and J. Huertas-Lozano. (*J. Pharmacol.*, 1948, **93**, 223.) Niaara, an arrow-poison from Colombia, is the latex of the tree "Pacuru-niaara," or "poison tree," *Ogcodeia ternstroemiiflora* Midbr. A white, amorphous principle, niaarin, having chemical and pharmacological properties characteristic of the cardiac glycosides, has been isolated from this latex. The intravenous LD50 in cats is 0·21 mg./kg.; it is poorly absorbed from the gastro-intestinal tract. Seven cases of congestive heart failure in man were successfully treated with niaarin, injected intravenously daily in doses of 0·50 to 0·75 mg. for the first two days, and then 0·25 mg. daily for a further 2 or 3 days. The therapeutic effect was evident within less than 24 hours after administration. On the whole, niaarin compares closely with strophanthin in rapidity and brevity of action. Cumulation is not important when 0·25 mg. is given as the daily maintenance dose. No side effects were noted in these cases, and no curare-like action is produced. Although niaarin is an effective therapeutic agent, the necessity for intravenous administration limits its field of usefulness. On the other hand, the actions of niaarin develop with remarkable rapidity which may occasionally be desired. S. L. W.

Ouabagenin. K. K. Chen, R. C. Anderson and H. M. Worth. (*J. Pharmacol.*, 1948, **93**, 156.) Employing a crystalline form of ouabagenin, the authors determined it to be approximately one-half as active on the heart as ouabain in cats and about one-third as active in frogs. It is more effective than ouabain in causing vomiting of non-anæsthetised cats, weight for weight, and the emetic dose is therefore no measure of the cardiac activity when different compounds are compared. Unlike digitoxigenin, it does not cause convulsions in cats or frogs. S. L. W.

Penicillin Treatment of Nasopharyngeal Diphtheria. E. W. Bixby. (*Amer. J. med. Sci.*, 1948, **215**, 509.) A series of 139 cases of diphtheria in young men, all of whom had been previously immunised according to the U.S. Army Schedule, were selected for study.. Although the typical grey membrane was seldom found, each case included in the report had a positive nasal and/or pharyngeal culture for *Corynebacterium diphtheriæ*, and gave a confirmed positive virulence study. Every case received 100,000 units of diptheria antitoxin intramuscularly, and general routine treatment. Of the 88 cases receiving only this treatment 53 (60 per cent.) developed a carrier state after two weeks. To the remaining 51 cases, penicillin was administered intramuscularly immediately on confirmation of the presence of *C. diphtheriæ*, in doses of 20,000 units every 3 hours for 50 doses. Of these, 43 (79 per cent.) had permanently negative cultures after 2 weeks. The average time required in hospital by patients with a 2-week negative culture was 35 days compared with an average of 50 days for those cases with a two-week

positive culture. In contrast to these results, the use of penicillin later in 40 cases of the two-week positive group who had previously received only the routine treatment was not successful, 60 per cent. remaining positive. The incidence of post-diphtheritic complications was not affected by penicillin although the severity of complications was modified. H. T. B.

Sodium Salicylate, Cutaneous Absorption of. G. Valette and R. César. (*Ann. pharm. Franc.*, 1948, **6**, 16.) The object of this investigation was to apply the characteristic elimination of sodium salicylate in the urine, to the study of the factors governing the absorption by the skin of non-liposoluble substances. The addition of eucalyptol markedly enhances the cutaneous penetration of sodium salicylate; the degree of penetration is dependent on the proportion of eucalyptol, but when the proportion exceeds 20 per cent. of the volume of the solution it produces an irritant effect. Sodium salicylate is hydrolysed in solution; when eucalyptol is added to such a solution it dissolves the salicylic acid liberated, with which it gradually becomes charged and penetrates the skin, and the irritation observed is thus attributable not to the eucalyptol but to the salicylic acid dissolved in it. This action of eucalyptol is less marked in alcoholic than in aqueous solutions. The addition of an emulsifying agent to a mixture of eucalyptol and solution of sodium salicylate does not increase the effect produced, though the results are better with oil-in-water than with water-in-oil emulsions. The renal excretion of sodium salicylate after cutaneous application of an aqueous solution containing eucalyptol, follows almost the same rhythm as after administration of the salt orally or subcutaneously, elimination reaching its maximum in 3 hours and continuing for about 48 hours. The alkalisation of solutions of sodium salicylate (to *p*H 8·4) was found to hinder the cutaneous penetration of the salt, while acidification (to *p*H 4·6) was found to increase it. S. L. W.

Sulphetrone, Pharmacology and Chemotherapy of. G. Brownlee, A. F. Green and M. Woodbine. (*Brit. J. Pharmacol.*, 1948, **3**, 15.) Sulphetrone is 4 : 4′-*bis*(γphenyl-*n*-propyl-amino)diphenylsulphone-tetrasodium sulphonate, an amorphous material containing, when air-dried, from 5 to 7 per cent. of water. It is insoluble in alcohol and other organic solvents, but is exceedingly soluble in cold water to give a syrup; 20 and 40 per cent. w/v solutions are stable when neutral or slightly alkaline, and may be autoclaved. A 10 per cent. w/v solution is isotonic with 0·91 per cent. sodium chloride solution, and hypertonic solutions up to 60 per cent. are readily obtained. In mice and dogs sulphetrone has an acute toxicity many times less than that of sulphanilamide. Very large doses can be given by mouth to mice and dogs without producing symptoms or pathological changes, but similar doses in rabbits produce anæmia. It causes hyperæmia and hyperplasia of the thyroid gland. Sulphetrone is not hydrolysed to diaminodiphenylsulphone in the body. When given orally or parenterally, it raises the alkali reserve of the plasma in the rabbit and the dog, but over a period of time, equilibrium is established. When given by mouth not only is sulphetrone the least toxic of the sulphones, but is also less toxic than any of the sulphonamides. It is only slowly absorbed from the intestinal tract; when given intravenously it is excreted in the urine almost completely in 24 hours, but when given orally only 75 per cent. is excreted in the same time. The drug is conjugated in the experimental animal or in man. It penetrates all tissues with extreme rapidity, with the exception of the brain, and is present in them in about the same concentration as in blood, but it enters the cerebrospinal fluid rather more slowly than do other sulphonamides. It has no action

on smooth muscle, heart, blood pressure or respiration. Antibacterial *in vitro* studies show sulphetrone to approach the efficiency of diaminodiphenylsulphone against avian, bovine and human strains of *Mycobacterium tuberculosis;* blood from guinea-pigs which had previously received parenteral sulphretone inhibits *in vitro* strains of virulent mycobacteria. The authors conclude that sulphetrone may prove effective in the treatment of experimental tuberculosis in laboratory animals, and that its administration to man in large doses for protracted periods is a practical possibility. S. L. W.

Sulphetrone, Treatment of Tuberculosis with. D. G. Madigan. (*Lancet*, 1948, **255**, 174.) In 70 cases of tuberculosis affecting different organs, sulphetrone was given for periods varying from a few days in tuberculous meningitis to eighteen months in more chronic cases. A blood-sulphetrone level of 7·5 to 10 mg./100 ml. should be aimed at by a scheme of gradual dosage. A suitable initial dose for adults is 1·5 g. daily (0·5 g. eight-hourly) for the first week, and 3 g. daily (0·5 g. four-hourly) for the second, the daily dose then being increased by 1 to 2 g. each week until the required blood level is reached, usually with 6 to 10 g. daily. If given intramuscularly combined with streptomycin, for miliary tuberculosis or tuberculous meningitis, 0·05 g./kg. of bodyweight every 4 to 6 hours for the first 24 hours is suitable, increased to 0·1 g./kg. during the second 24 hours; when the meninges are involved, levels above 5 mg./100 ml. may cause vomiting. It is essential to give iron and brewers' yeast for a fortnight before and throughout sulphetrone treatment to avoid hypochromic and nutritional anæmia; the yeast also prevents the onset of peripheral neuritis, occasionally seen. Even so, a residual hæmolytic anæmia develops and continues throughout treatment leading to a fall in hæmoglobin content to a level as low as 60 per cent.; should it fall below this figure the sulphetrone should be withdrawn. Besides a weekly check of red cells and hæmoglobin, there should be a weekly estimate of blood-sulphetrone, which should not exceed 12·5 mg./100 ml. Danger signals are continuous headache, loss of appetite, nausea and vomiting, gastro-intestinal discomfort, dizziness and mental confusion; these conditions are associated with high blood-sulphetrone levels and measures should be taken to hasten elimination of sulphetrone by giving fluids by all routes. Stasis also should always be guarded against. In general, no beneficial effect was detected from sulphetrone therapy of acute infections, but improvement was observed in chronic lesions. Thus, 12 out of 17 cases of acute pulmonary fibrocaseous disease, and 13 out of 22 chronic cases improved. All of 4 cases of primary pulmonary tuberculosis, and 6 out of 8 strictly exudative lesions, improved. All of 4 in the chronic hæmatogenous group and 3 out of 4 in the productive pulmonary infiltrative group improved. In general, all exudative phases of infiltrative disease were halted and reversed by sulphetrone. The need for long-continued courses is emphasised and routine laboratory control is essential. Sulphetrone is useful as an adjuvant with definite objectives in view. S. L. W.

Thenylene: A New Antihistamine Compound. A. S. Friedlaender and S. Friedlaender. (*Amer. J. med. Sci.*, 1948, **215**, 531.) A new antihistamine compound, N-(α-pyridyl)-N-(α-thenyl)-N′, N′-dimethylethylenediamine hydrochloride, has been synthesised. Under the name of thenylene (of histadyl) it has been examined to ascertain its effectiveness in preventing fatal histamine shock and anaphylaxis in guinea-pigs, and in alleviating allergic symptoms in man. A protective dose of 3 mg./kg. was administered intraperitoneally to male guinea-pigs 15 minutes prior to the intravenous injection of histamine. This dose protected all animals against one lethal

dose of histamine, while 50 per cent. survived approximately 8 lethal doses. Marked protection was given against fatal anaphylaxis in guinea-pigs sensitised by the intraperitoneal injection of 0·1 ml. of normal horse serum, followed 12 days later by a shock dose of 1 ml. of the same serum intravenously. Of the control animals 100 per cent. died, while only 20 per cent. of fatalities occurred amongst a group receiving 1 mg./kg. 15 minutes before the dose of antigen. The clinical action was studied in 117 patients with one or more allergic complaints, in doses of 100 mg. for adults 4 times daily, or as necessary when symptoms were intermittent. Children were given one-quarter to one-half this dosage. Symptomatic relief was obtained in many cases of urticaria, hay fever and perennial allergic rhinitis. Results in asthma were not striking. Mild side effects occurred in 25 per cent. of patients, but rarely affected administration of the drug. Drowsiness was most common, occurring in 13 patients, and vertigo, headache, gastro-intestinal distress and dryness of mucous membranes were also reported. Toxic symptoms were usually relieved by a reduction of dosage. H. T. B.

BACTERIOLOGY AND CLINICAL TESTS

Antibiotics, Induced Resistance of *Staphylococcus aureus* to. J. W. Klimek, C. J. Cavallito and J. H. Bailey. (*J. Bact.*, 1948, **55**, 139.) It is known that many antibacterial substances are inactivated by various thiol compounds, some reacting rapidly with a large number of -SH compounds, some with cysteine or related β-aminoalkane thiols only, and others displaying reactions intermediate between these two. A study of the development of resistance of *Staphylococcus aureus* to several antibiotics was undertaken to determine whether a correlation existed between development of resistance and the known reactivity of the antibiotics with thiol compounds. The antibacterial agents studied were penicillin, streptomycin, pyocyanin, gliotoxin, aspergillic acid, mercuric chloride, and the active principles of *Allium sativum*, *Asarum canadense* and *Arctium minus*. The susceptibility of *S. aureus* to the antibiotics was determined by growing the organism in a series of beef broth cultures containing increasing quantities of the test agents, Results of the experiments are demonstrated in figures, and show the ability of *S. aureus* to develop rapid and marked resistance to penicillin, streptomycin and the active principle of *Asarum canadense*. The organism developed an intermediate degree of resistance to pyocyanin and gliotoxin, very little resistance to mercuric chloride or the active principle of *Arctium minus* and no resistance to aspergillic acid. These results run parallel with the degree of specificity of reactivity with sulphydryl groups. The more selective the antibiotic as to the type of -SH compound with which it will react, the more readily does it induce bacterial resistance. Reversibility of resistance occurs with antibiotics which react non-selectively with thiols, while the resistance induced by antibiotics reacting selectively with thiols is likely to be non-reversible. H. T. B.

Streptomycin. Activity in Presence of Serum and Blood. E. B. Schoenbach and C. A. Chandler. (*Proc. Soc. exp. Biol. N.Y.*, 1947, **66**, 493.) Bactericidal tests were carried out on the growth of *Staphylococcus aureus* in the presence of streptomycin, various factors possibly affecting such tests, i.e. phagocytosis, hæmolysis, immune serum and labile

[*Continued on page 265*

PHARMACOPŒIAS AND FORMULARIES

SCANDINAVIAN PHARMACOPŒIA COMMISSION

At a Conference held, on the invitation of the Swedish Government, at Stockholm in November 1948, a joint Pharmacopœia Commission for the Scandinavian countries was formed. This consists of 3 representatives of each of the Pharmacopœia Commissions of Denmark, Norway and Sweden, nominated by the Governments of these countries. It is hoped that Finland will also join. The chairman is Prof. G. Ahlgren, Sweden, and the General Secretary, Dr. F. Reimers, Denmark.

Formation of the joint commission has given a permanent organisation to the co-operation between the Pharmacopœia Commissions of the Scandinavian countries which has been kept up by occasional conferences throughout a number of years. While, in the earlier period of co-operation, it was only possible to obtain agreement on single points, work will now be directed towards gradually obtaining such complete agreement that a joint Scandinavian Pharmacopœia can be published.

That would have many advantages. The medical and pharmaceutical professions are so close to one another in these countries, and the difference in language is so small, that any difference between the Pharmacopœias should be avoided, as this may prove a hindrance in connection with scientific literature, text-books, and education, and also may give rise to difficulties in understanding prescriptions written by doctors of the other countries. Further, all the countries manufacture chemicals to a limited extent only and most of the requirements are imported from the same suppliers abroad. It will also be a great advantage that work can be shared among the Pharmacopœical laboratories of these countries, for initial attack on problems and for checking of results.

Instructions to the joint commission have been drafted and are under consideration by the respective Governments. One important decision is that the names of drugs to be used in the National Pharmacopœias shall be decided by the joint Commission.

F. REIMERS.

ABSTRACTS (*continued from page 264*)

constituents being also investigated. A susceptible strain of the organism was used and also a variant of that strain resistant to 1,000 μg. of streptomycin. In broth, the bacteriostatic range of streptomycin for the susceptible strain was not affected by the presence of the 60 per cent. of human or rabbit serum. When the resistant strain was tested in the presence of 60 per cent. of rabbit serum, as little as 125 μg./ml. of streptomycin gave retarded growth with small inocula at 24 hours but not at 48 hours. This transient inhibition was not due to any constituent of the serum, neither was it due to stimulation of phagocytosis with subsequent death of the leucocytes. Possibly the inhibition is due to modification of the nutritional requirements with the acquisition of streptomycin resistance. It is suggested that there are two alternative growth mechanisms available to the organism, one being blocked by streptomycin, the other being insufficiently developed at first but increasing later.

H. T. B.

BOOK REVIEWS

THE STUFF WE'RE MADE OF, by W. O. Kermack and P. Eggleton. (Pp. 356, 8 Plates, 75 Figs. in the Text.) E. Arnold and Co., London, 1948, 10s. 6d.

The second edition of this book is not greatly different from the first, since research during the last eight years has not seriously modified the facts and theories generally accepted before the first edition was published Additional information has accumulated, but mostly on detail outside the scope of this book. Knowledge of the vitamins has, however, grown so much that the one chapter devoted to them in the first edition has been replaced by two, one being devoted to the B vitamins. The parts played by riboflavine, nicotinic acid, pyridoxine, biotin, etc., in the nutrition of animals have been worked out, and more experiments have been performed on human beings who have volunteered for various dietary treatments. The production of certain factors by the bacteria present in the alimentary tract has been demonstrated and their worth to the animal noted. The destruction or inhibition of these valuable bacteria by drugs given for other purposes has also been shown. The other fresh chapter in this edition deals with the subject of muscle contraction. The action of adenine triphosphate on myosin and *vice versa* forms the basis for a theory of muscle contraction which the writers "make to work" by means of a working model built up of springs and "Lazy-tongs." They end this chapter with the sentence "Nature's engines are indeed miracles of design and construction; their existence would be incredible if they were not so utterly commonplace." The book is full of sound information, reasoned speculation and a way of "looking at the wood and not only at the trees." It is, in fact, so good that the present reviewer's only regret is that in so many places, the writers have tried to make the book "popular" by using frivolous and skittish expressions: e.g., the term "vitamin racket" has been applied to the whole study of vitamins. If it had been applied to the attempt to get anybody and everybody to dose himself liberally with synthetic vitamins, at a high cost in shillings or dollars, no one could have objected to the term. However, the fact that a second edition has been called for is evidence that the style of the writing has been acceptable to the general public for whom it was intended, and who would possibly not have read the book if it had been consistently serious all through.

K. H. COWARD.

THE PRESENTATION OF TECHNICAL INFORMATION, by R. O. Kapp. Pp. 140 and Index, Constable and Co., London, 1948, 6s.

The many problems arising in the writing of good technical English are carefully analysed in this book and much sound advice is offered to writers of papers, reports and reviews. Although the author himself is concerned with the engineering field his remarks apply equally well to other branches of science. The book is based on a series of lectures given at University College, London, a year or so ago. Functional English, which is not to be confused with Basic English, is described and recommended as the language for scientific writings. Its essential purpose is the conveyance of new information, which may be factual, or argumentative, and infer or suggest new lines of thought. The author discusses the

problem not only from the writer's viewpoint but also from that of the reader. Thus, in addition to being good English, Functional English must be easily readable. A good introduction with a statement of the terms of reference, a logical arrangement of thought and argument, the selection of material for its relevance to the problem in hand and the presentation of facts at a pace at which they can be understood and remembered are just as important as well-constructed sentences. A well-written report should not only give information, but should also stimulate thought in the reader. There are useful chapters on the avoidance of circumlocutions and the use of generalisations, qualifications and metaphor. In writing this book the author has put his own principles into practice and the result is a well-written, interesting and stimulating publication which should be read by both students and post-graduate workers. The adoption by students of Professor Kapp's suggestions at an early stage would go far towards the production of laboratory notes of the high standard spoken of so frequently by examiners.

BOOKS RECEIVED

THE CHEMICAL FORMULARY by H. Bennett, Vol. VIII. Pp. 428 and Index, Chapman and Hall, Ltd., London, 1948, 42s.

PRINCIPLES OF BIOLOGICAL ASSAY by C. W. Emmens. Pp. 204, Chapman and Hall, Ltd., London, 1948, 21s.

THE BACKGROUND OF THERAPEUTICS by J. H. Burn. Pp. 335 and Index, Oxford Medical Publications, London, 1948, 22s. 6d.

GRUNDLAGEN DER PHARMAKOLOGIE by K. W. Merz. Pp. 274 and Index, Wissenschaftliche Verlagsgesellschaft M.B.H., Stuttgart, 1948, 4th Ed.

PRECIS DE CHIMIE TOXICOLOGIQUE by F. Schoofs. Pp. 513 and Index, Les Presses Universitaires de Liège, 1948, 2nd ed.

LA CHIMIE DES VITAMINES ET DES HORMONES by J. Sivadjin, Vol. I. Pp. 479, Gauthier-Villars, Paris, 1948, 3rd ed.

PRACTICAL PHARMACOGNOSY by B. E. Hébert and K. W. Ellery. Pp. 365 and Index, Ballière, Tindall and Cox, London, 1948, 21s.

TRACE ELEMENTS IN FOOD by G. W. Monier Williams. Pp. 498 and Index, Chapman and Hall, Ltd., London, 1949, 30s.

LETTERS TO THE EDITOR

The Specific Rotation of Emetine Hydrochloride

SIR,—The following observations, which seem of sufficient interest to record, were made when we were asked to examine a sample of emetine hydrochloride according to the standard of the French Codex (6th edition, 1937).

Emetine hydrochloride of the French Codex is required to have a specific rotation of $+53°$ when determined on a 2 per cent. solution of the anhydrous salt in chloroform but, under these conditions and using chloroform B.P. as solvent, our specimen had $[\alpha]_D^{20°C.} + 47{\cdot}5°$. It is noteworthy that Carr and Pyman[1] give $+53°$ as the specific rotation for the anhydrous salt in chloroform.

As is well known, pure chloroform is unstable and for this reason it is customary to add a small percentage of alcohol as preservative; thus chloroform of the B.P. is required to contain 1 to 2 per cent. v/v. of alcohol. It occurred to us that the alcohol content of the chloroform used as solvent might influence the specific rotation of emetine hydrochloride. Accordingly, we prepared some pure chloroform, free from alcohol, and determined the specific rotations of three batches of emetine hydrochloride, dried *in vacuo* over phosphorus pentoxide for 48 hours, in this solvent. The results for $[\alpha]_D^{20°C.}$ for 2 per cent. solutions of these samples in pure chloroform were: (1) $+59.64°$, (2) $59{\cdot}98°$ and (3) $59.98°$. A sample of chloroform B.P. was then prepared by adding 1·5 per cent. v/v. of absolute alcohol to our pure chloroform and the specific rotation of anhydrous emetine hydrochloride (sample 1) determined in this solvent. The figure obtained was $+47{\cdot}3°$ (c, 2·0).

It is evident that the actual figure for the specific rotation is greatly influenced by the alcohol content of the chloroform used as solvent and, on this account, we believe that it is more reliable to carry out the determination with an aqueous solution. In our experience, emetine hydrochloride has $[\alpha]_D^{20°C.} + 17{\cdot}8°$ when determined on an accurately prepared 5 per cent. w/v. solution of the anhydrous salt in water. Indeed, we have examined hundreds of samples in aqueous solution and the specific rotations have never deviated from $+17{\cdot}8°$ by more than a few tenths of a degree. Further evidence of the erratic results, which may be obtained when using chloroform as solvent, was afforded when we decided to repeat our work a week after the original experiments. Our pure chloroform had then become slightly acid in reaction owing to decomposition. The specific rotation of anhydrous emetine hydrochloride in this solvent was $+72{\cdot}86°$, but after purification of the chloroform a figure of $+59{\cdot}81°$ was obtained.

The Wellcome Chemical Works, Dartford.
January 27, 1949.

A. E. BEESLEY.
G. E. FOSTER.

REFERENCE

1. Carr and Pyman, *J. chem. Soc.*, 1914, **105**, 1604.

NEW REMEDIES

The asterisk (*) after the name of an article indicates that the information given is derived from the makers' publications. Further information regarding these articles may be obtained by application to the Editor.

Abidex Drops* are a stable, non-oily and non-alcoholic multivitamin solution intended for the prevention and treatment of vitamin deficiencies, particularly in children. Each 10 min. (30 drops) contains: vitamin A 5000 I.U., vitamin D 1000 I.U., aneurine hydrochloride 1 mg., riboflavine 0·4 mg., nicotinamide 5 mg., ascorbic acid 25 mg. The drops are well tolerated by infants and children, and may be taken directly or mixed with milk, fruit juice, soup or other foods; they may also be safely added to the contents of the infant's feeding-bottle. The average daily dose for infants under 1 year is 5 min., and for older children 10 min. The drops are supplied in 10-ml. bottles, with a dropper. S. L. W.

Beplex Elixir* is an aqueous extract of rice bran with members of the vitamin B complex added so that each ml. contains: aneurine hydrochloride 0·125 mg., riboflavine 0·25 mg., nicotinic acid 1·25 mg., calcium pantothenate 0·625 mg., together with significant amounts of choline, inositol and other factors; it also contains 16 per cent. of alcohol. The dose is 2 fl. dr. daily, or as prescribed. Beplex capsules contain: aneurine hydrochloride 1 mg., riboflavine 0·80 mg., nicotinamide 10 mg., pyridoxine 0·012 mg., and pantothenic acid 0·013 mg. The dose is 3 or more capsules daily. The elixir is issued in 4-ounce bottles and the capsules in bottles of 50. S. L. W.

Bismuth Sodium Triglycollamate. (*New and Non-official Remedies, J. Amer. Med. Ass.*, 1948, **138,** 749.) Bismuth sodium triglycollamate ($C_{24}H_{28}O_{25}N_4BiNa_7$) is a double salt of bismuthyl sodium triglycollamate and disodium triglycollamate, containing about 18·3 per cent. of Bi. It is a white, odourless, stable, crystalline powder with a saline taste, soluble in water and insoluble in organic solvents. It must comply with limit tests for carbonate, chloride, sulphate, nitrate, lead, copper, silver, arsenic and moisture. It is assayed by igniting at 700°C., precipitating the bismuth with hydrogen sulphide and weighing the bismuth sulphide obtained. Bismuth sodium triglycollamate is used for the oral administration of bismuth in the treatment of syphilis, alone, or in conjunction with other antisyphilitics. It has the same contraindications as other bismuth preparations. The total daily dose is 0·82 g. (equivalent to about 150 mg. of bismuth) to 1·23 g. (225 mg. of bismuth) given in 2 or 3 divided doses. It is supplied in the U.S.A. as tablets containing 0·41 g. (75 mg. of bismuth) under the trade-name Bistrimate. G. R. K.

Dermogesic Ointment* contains, in each 100 g., calamine 8 g., benzocaine 3 g., and hexylated metacresol 0·05 g., in a vanishing cream base. It is a bland, non-greasy, analgesic ointment for the relief of irritant skin conditions. It is supplied in 1 oz. collapsible tubes. S. L. W.

Dihydrocodeinone Bitartrate. (*New and Non-official Remedies, J. Amer. med. Ass.*, 1948, **138,** 820.) Dihydrocodeinone bitartrate ($C_{18}H_{21}O_2N$, $C_4H_6O_6$, $2\frac{1}{2}H_2O$) is the hydrated bitartrate of dihydrocodeinone, and occurs as a white, odourless, crystalline powder, soluble in water and slightly soluble in alcohol: a 5 per cent. aqueous solution has *p*H about 3·5. It is identified by the m. pt. of the base and the oxime, and is distinguished from morphine

by treatment with a solution of selenious acid in sulphuric acid ; dihydrocodeinone gives a green colour, which changes to blue and then slowly to purple, whereas morphine gives a blue colour, which changes to green and then to brown. Dihydrocodeinone bitartrate in sulphuric acid solution gives no colour with ferric chloride (distinction from codeine). It is assayed by dissolving the precipitated base in excess of sulphuric acid and titrating back with sodium hydroxide. It has an action similar to that of codeine, but weight for weight is more active and more liable to cause addiction. It is used to allay cough in the same manner as codeine, but it has no clear-cut advantage over the latter. The adult dose is 5 to 15 mg. given 3 or 4 times in 24 hours; children of 2 years and over may be given half the adult dose and younger children one quarter the adult dose. It is supplied in the U.S.A. under the trade-name " Hycodan." G. R .K.

Isobornyl Thiocyanoacetate,-Technical. (*New and Non-official Remedies*, *J. Amer. med. Ass.*, 1948, **136,** 1099.) Isobornyl thiocyanoacetate contains 82 per cent. or more of $C_{13}H_{19}ON_2S$, mol. wt. 253·35, with other terpenes. It is a yellow, oily liquid; odour terpene-like; very soluble in alcohol, benzene, chloroform and in ether, practically insoluble in water. Refractive index, 1·512; weight per ml. at 20°C., 1·1465 g.; acid number, 1·19. When 5 ml. of 2N alcoholic potassium hydroxide is added to 25 mg. of isobornyl thiocyanoacetate-technical and the solution heated for 5 minutes acidified with diluted sulphuric acid, and a few drops of ferric ammonium sulphate test solution added, a red colour develops. When 1 ml. of a 10 per cent. w/v test-solution of ferrous sulphate is added to the heated mixture of isobornyl thiocyanoacetate and alcoholic potassium hydroxide and the solution is warmed for another 5 minutes and acidified with diluted sulphuric acid, a blue colour develops. On adding 1 ml. of 2N alcoholic potassium hydroxide to a 5 ml. of a 10 per cent. alcoholic solution, a yellow colour, which rapidly changes to deep orange, is formed. Isoborneol crystals, m.pt. 200° to 205°C., are obtained by hydrolysis with potassium hydroxide. Nitrogen, by the Kjedldahl method, should not be less than 4.6 per cent., which is equivalent to an isobornyl thiocyanoacetate content of 80 per cent. An oily emulsion containing 5 per cent. isobornyl thiocyanoacetate-technical and 0·6 per cent. of dioctyl sodium sulphosuccinate, is an effective pediculicide. L. H. P.

Myanesin* is a proprietary brand of $\alpha:\beta$-dihydroxy-γ-(2-methylphenoxy)-propane, and is the most effective member of a series of compounds possessing muscle-relaxing properties. It acts centrally by diminishing the reflex excitability of the spinal cord, and not on the myoneural junction as does curare. It does not act on the higher centres, and consciousness is not affected. Even in paralysing doses it does not produce respiratory arrest. Its use is indicated whenever complete relaxation of the abdominal musculature is required surgically without resorting to a deep plane of anæsthesia. It is suitable for use with any general anæsthetic or combination of anæsthetics, and is administered intravenously, usually in doses of 5 to 10 ml. of a 10 per cent. solution; the injection is given slowly 1 or 2 minutes before relaxation is required, and the effect of the dose lasts for 20 to 30 minutes. In patients with impaired renal function, or where a prolonged operation is being carried out, it is best administered as a 2 per cent. solution (prepared by the addition of the contents of a 10 ml. ampoule to 40 ml. of normal saline). Myanesin is issued in boxes containing 3 or 12 ampoules each containing 10 ml. of 10 per cent. solution.

S. L. W.

Panlittol* tablets contain 2½ grains of pancreatic extract and 1/10 grain of thyroid (B.P. 1932). The tablets are recommended for the control of essential hypertension, and in the treatment of disorders such as Raynaud's syndrome in which there is peripheral vascular spasm. The recommended initial dosage is 1 tablet 3 times daily, taken half an hour before meals: if necessary, the dose may be increased to 2 or 3 tablets 3 times daily. The administration of the tablets should be combined with the routine measures for the care of the hypertensive patient. Panlittol tablets are issued in bottles of 24, 100, 500 and 1,000. S. L. W.

Phytodermine* cream and powder are preparations for the treatment and prevention of fungous infections of the skin, particularly athlete's foot. The cream, which is applied to the affected part at night after bathing, contains phenylmercuric acetate 0·167 per cent., terpineol 1 per cent. and salicylic acid 3 per cent. in a water-miscible base. The powder, which is dusted into socks and shoes in the morning, contains methyl *para*hydroxybenzoate 5 per cent., salicylic acid 5 per cent. and perfumed talc 90 per cent. S. L. W.

Promanide* is pp′-diaminodiphenylsulphone-N,N′-di-dextrose sodium sulphonate (promin) in the form of a jelly containing 5 per cent. in a water-soluble tragacanth base, for topical application in the treatment of accessible tuberculous lesions, or of a 5 per cent. water-soluble ointment for surface application in conditions such as the ulcerative type of lupus. From 3 to 10 ml. of the jelly or ointment may be applied 2 or 3 times a week or more often, or the jelly may be injected into the abscess or sinus. Promanide jelly and ointment are supplied in 2-oz. jars. S. L. W.

Promin* is *pp′*-diaminodiphenylsulphone-N,N′-di-dextrose sodium sulphonate and is employed by intravenous injection in the treatment of leprosy. The treatment must be continued over a period of many months, the average intravenous dose being from 2 to 5 g. (from 5 to 12·5 ml. of promin solution), administered daily for 6 consecutive days and omitted on the seventh, with an interval of 1 week at the conclusion of each 2 weeks' treatment. Serious toxic reactions are rare, but patients should be under constant observation and blood counts taken every 2 weeks. Encouraging results are also claimed for the use of promin in tuberculosis. It is not a sterilising drug in tuberculosis, and the terminal stages of the disease are not improved, but arrest or temporary stabilisation of the disease can be expected in selected cases of early non-destructive lesions. The best results have been obtained in the preparation of patients for surgical procedure. Promin is supplied in sterile aqueous solution in 5 ml. ampoules containing 2 g. and in 12·5 ml. ampoules containing 5 g. It is issued in boxes of 25 ampoules. S. L. W.

***d*-Tubocurarine Chloride.** (*New and Non-official Remedies, J. Amer. med. Ass.*, 1948, **138**, 821.) *d*-Tubocurarine chloride ($C_{38}H_{44}O_6N_2C_{l2}$, $5H_2O$) is the crystalline chloride of a quaternary base alkaloid obtainable from the bark and stems of *Chondodendron tomentosum* and related species (a tentative structural formula is given). It is a colourless or yellowish-white to grey or light brown, odourless, crystalline powder, soluble in water, slightly soluble in alcohol, and almost insoluble in chloroform and ether; m. pt. about 265° to 278°C. A dilute solution gives a brilliant blue colour when treated with Folin-Ciocalteu phenol reagent and sodium carbonate and heated in a water-bath. Other identification tests depend upon the production of a pink

(Continued on page 272)

NEW APPARATUS

APPARATUS FOR FILTRATION UNDER REDUCED PRESSURE

By E. D. Banks

From the Analytical Control Laboratories, May & Baker, Ltd.

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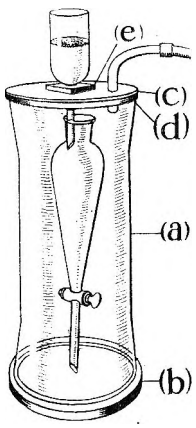

During analytical operations, in order to avoid quantitative transference of a filtrate and the further washing involved, it is frequently advantageous to filter under reduced pressure into a vessel not made to withstand it. The apparatus described, which can be used for direct filtration into various sizes of beakers, separators, basins, flasks, etc., is simple and readily constructed. The metal parts are easily made in the laboratory workshop, while the glass tubing and glass discs are standard items which can be obtained from suppliers of chemical glassware. The apparatus consists of a standard 12-in length of 4-in. diameter butt-ended Pyrex tubing (a) of approximately $\frac{1}{8}$-in. wall sealed at the bottom with a 6-in. glass inspection-disc (b) cemented with Canada balsam or other suitable adhesive. The top plate (c) is made from a 6-in. diameter metal disc about $\frac{1}{8}$-in. thickness drilled with a central 1-in. hole and fitted with a $\frac{1}{4}$-in. diameter tube for connection to the pump brazed half-way between the centre and circumference. A rubber washer (d) forms a gasket between the plate and glass tubing and a second thick rubber washer (e) ensures an air-tight junction between the filter funnel or Gooch adaptor and the metal disc. Using the above dimensions filtration is possible into graduated flasks up to 500 ml. and separators up to 250 ml. capacity. Smaller apparatus may be raised to a convenient height on wooden blocks.

NEW REMEDIES (*continued from page 271*)

colour with Reinecke's salt and a yellow colour with trinitrophenol and with sulphuric acid and potassium iodate. *d*-Tubocurarine chloride, dried at 100°C. for 4 hours, loses not more than 11·5 per cent. of its weight, and contains 9·5 to 10·2 per cent. of chlorine, calculated on the dried material. The weight of the residue obtained by treating an aqueous solution with sodium bicarbonate, extracting with chloroform, removing the chloroform and drying is not more than 3 per cent., calculated on the dried material, and the residue itself is insoluble in water, but soluble in dilute hydrochloric acid. *d*-Tubocurarine chloride has a specific rotation in 1 per cent. w/v solution of +208° to +217° (the most probable value for pure anhydrous *d*-tubocurarine chloride is +215°). It is standardised biologically by the rabbit "head-drop" method; the standard "head-drop" dose HD50, calculated as $C_{38}H_{44}O_6N_2Cl_2$, $5H_2O$, is 0·15 mg./kg. of body weight, and references to the method of assay are given. *d*-Tubocurarine chloride is used to reduce the tone or contractile power of skeletal muscle in anæsthesia, shock therapy, and in certain spastic states. It is also used for the diagnosis of myasthenia gravis.

G. R. K.

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