SYNTHESIS EMPLOYING ACETYLENIC PRECURSORS

Part 1 Synthesis of Histamine.

Part 2 Structure of Carpaine.

Part 3 Synthetic Studies on Baikiain.

Part 4 Synthesis of 2-Deoxy-D,L-ribose.

A THESIS FOR THE DEGREE OF PhD

by

Matthew M. Fraser. B.Sc..

Modderfontein,
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SUMMARY OF PH.D. THESIS

Matthew M. Fraser.

SYNTHESES EMPLOYING ACETYLENIC PRECURSORS.

A. Synthesis of Histamine. The allergenic agent histamine (4-2'-aminoethylglyoxaline) was prepared from but-2-yne-1:4-diol by a six-stage synthesis in high overall yield (41%).

B. Structure of Carpaine. A key degradation product of the alkaloid carpaine had been previously formulated as 9-hydroxy-9-methyltridecoic acid. This acid was synthesized by an unequivocal route and was found to possess physical and chemical properties radically different from those of the degradation product. This evidence confirms recent work that the structure of carpaine has been erroneously formulated.

C. Synthetic Studies on Baikiain. (1:2:3:6-Tetrahydropyridene-2-carboxylic acid). The amino acids 1:5-diamino-1-carboxypent-3(cis)-ene and 1-amino-1-carboxypent-3(cis)-en-5-ol were synthesised from but-2-yne-1:4-diol. It was expected that these amino acids would undergo ready ring closure, to produce baikiain, by elimination of ammonia and water respectively. All attempts at ring closure on the two amino acids, however, gave unchanged starting material or caused

decomposition.

D. Synthesis of 2-Deoxy-D,L-ribose. Starting from the commercially available but-2-yne-1:4-diol synthetic routes to this important sugar were extensively investigated. The best route was found to be via the intermediate D,L-erythro-1:4-(dicarbethoxyamino)pentane-3:4:5-triol, hydrolysee of which with mild alkali gave syrupy 2-deoxy-D,L-ribose.

ACKNOWLEDGEMENTS

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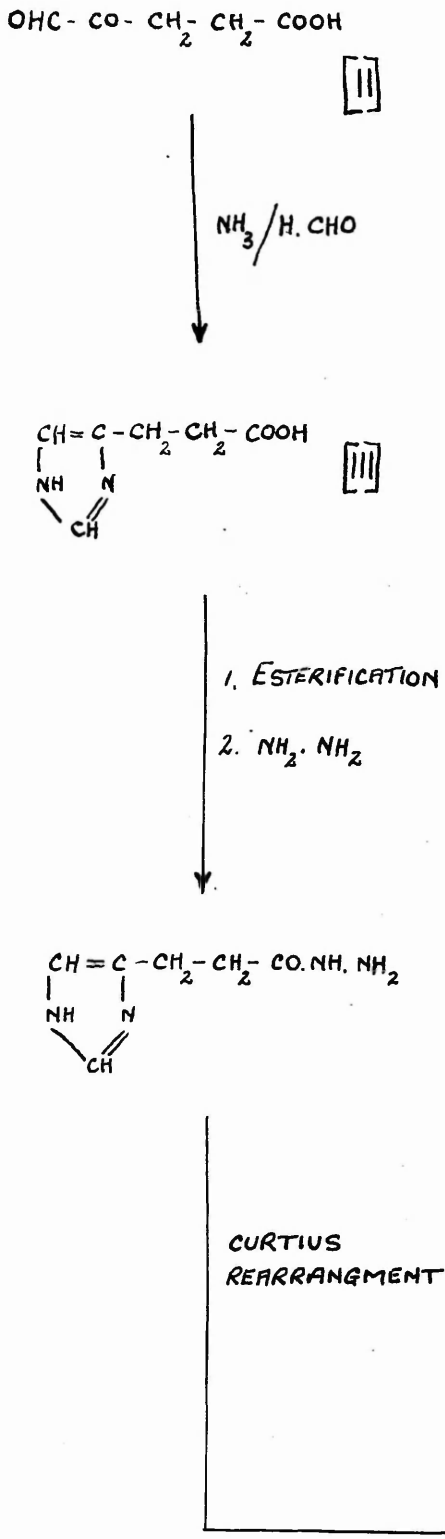
CONTENTS

PART 1

THE SYNTHESIS OF HISTAMINE

FLOW SHEET A

ROUTE A



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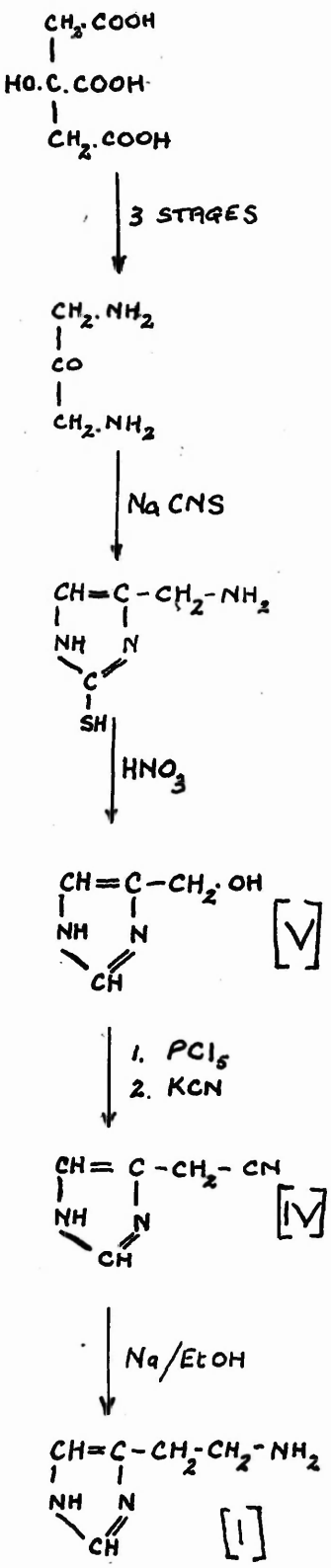

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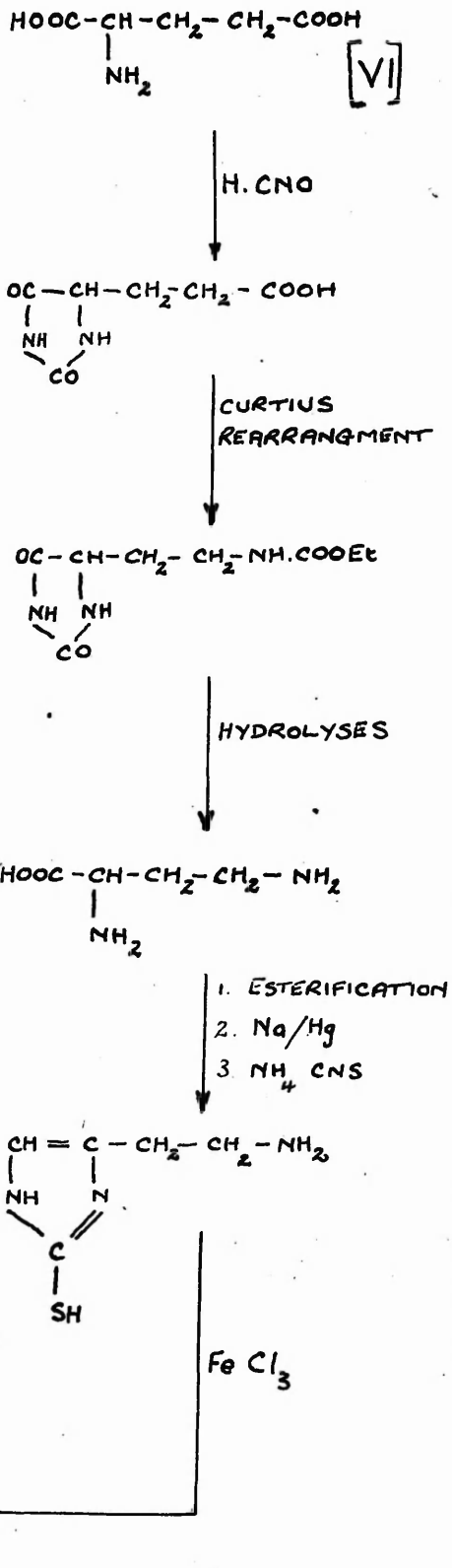


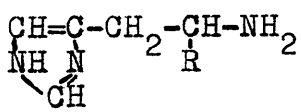
THE SYNTHESIS OF HISTAMINE

INDEX

HISTORICAL.

The allergenic agent histamine (4-2'-aminoethylglyoxaline) (1; R=H) has been shown to occur in most animal cells in which it appears to be held in an inactive form[1]. Its chief physiological effects involve the contraction of plain muscle, dilation of capillaries and stimulation of the suprarenal and certain stomach glands. In man the main sites of action of histamine are the skin, the mucous membranes and the bronchioles. It is interesting that the tissues most reactive to histamine are those in which the most intensive allergenic responses occur.

Anaphylaxis, a condition of supersensitivity to certain proteins, appears to involve the release of histamine from the body cells with consequent physiological action on the bronchioles and mucous membranes (asthma and hay fever) and on the skin (rashes etc.).



(1)

Histamine was first isolated from a natural source in 1910 by Barger and Dale [2] who obtained it from an aqueous extract of ergot, a fungus found on rye. After its separation they proved it identical with an amine prepared a year earlier by Ackermann[3]. This base had been prepared from the amino acid histidine (1; R=COOH) by bacterial decarboxylation.

The first synthesis of histamine was achieved in 1907, three years before its discovery in nature, by Windaus and Vogt[4] when investigating a possible synthetic route to histidine. Starting from β-glyoxylpropionic acid (11; Route A; Flow Sheet A; Opp. p. 1) they prepared 4-2'-carboxyethyl-glyoxaline (111) as described by Knoop and Windaus[5]; Curtius degradation of this product led to histamine. In some steps yields were not stated so that an overall yield cannot be given but as the starting material was inaccessible and expensive the method was mainly of academic interest.

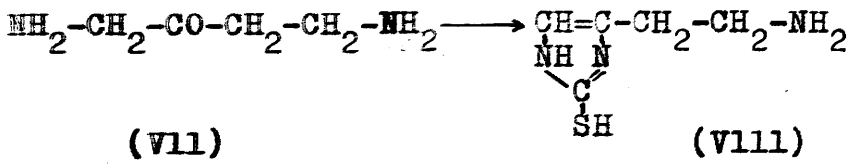
The first practical synthesis was by Pyman[6] in 1911, employing an eight-stage synthesis from citric acid (Route B; Flow Sheet A; Opp. p. 1). The overall yield was 1%. This method was improved a few years later by Koessler and Hanke[7] the overall yield being increased to 4%.

Meanwhile possible routes to histamine from histidine were being investigated. As Ackermanns' bacterial method gave uncertain yields Ewins and Pyman[8] in 1911 investigated other methods and eventually obtained 25% yields of histamine by heating histidine in sealed tubes with dilute acids. Following this, the discovery in 1916 by Dakin[9] that one of Pymans intermediates, 4-cyanomethylglyoxaline (1V; Route B), could be prepared in 80% yield by the action of chloramine T on histidine, made possible another commercial route to histamine. The more recent availability of another of Pymans intermediates, 4-hydroxymethylglyoxaline (V; Route B) from

fructose[10] and from 1:3-dichloroacetone and sorbose[11] has shortened the reaction sequence but th-e overall yield remains about 10%.

Another route to histamine was investigated by Abakori and Numano in 1936[12]. Their eight stage synthesis (Route C; Flow Sheet A; Opp. p. 1) starting from ∝-aminoglutaric acid (V1) had a 5% overall yield. This low conversion renders the synthesis inferior to Pymans general method.

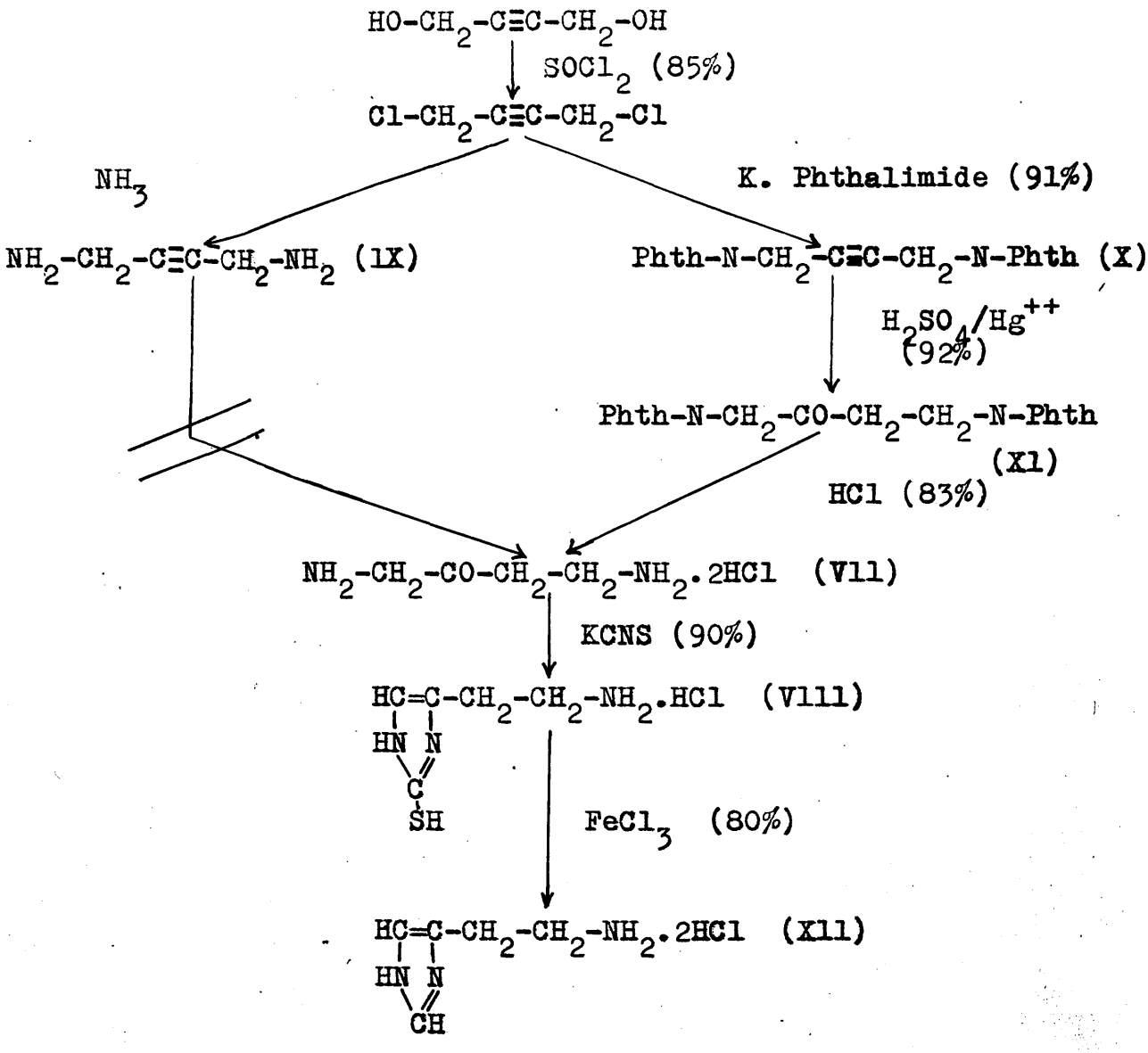
In view of the complexity of the above methods and the low yields obtained, a new approach to histamine seemed desirable. It was noted that in 1930 Pyman[13] had prepared histamine by condensing 1:4-diaminobutan-2-one (V11) with sodium thiocyanate and oxidising the resulting 2-mercapto-



(V11) (V111)

histamine (V111) with ferric chloride. This, however, did not constitute a synthesis as the initial 1:4-diaminobutan-2-one was obtained by degrading histamine itself[14].

FLOW SHEET B.


HO-CH2-C≡C-CH2-OH
SOCl2 (85%)
Cl-CH2-C≡C-CH2-Cl
NH3
K. Phthalimide (91%)
NH2-CH2-C≡C-CH2-NH2 (IX)
Phth-N-CH2-C≡C-CH2-N-Phth (X)
H2SO4/Hg++
(92%)
Phth-N-CH2-CO-CH2-CH2-N-Phth
(XI)
HCl (83%)
NH2-CH2-CO-CH2-CH2-NH2.2HCl (VII)
KCNS (90%)
HC=C-CH2-CH2-NH2.HCl (VIII)
HN N
C
SH
FeCl3 (80%)
HC=C-CH2-CH2-NH2.2HCl (XII)
HN N
CH

DISCUSSION.

But-2-yne-1:4-diol was converted into histamine by a six-stage synthesis (Flow Sheet B; Opp. p.4), the overall yield being 41%.(15)

But-2-yne-1:4-diol was converted by thionyl chloride into 1:4-dichlorobut-2-yne (1X; Flow Sheet B) as described by Johnson(16). This compound, as reported by Johnson, had a mild vesicant action on the skin. Contact with the dihalide, however, over a period of months produced severer symptons, even traces of the dihalide causing extensive blistering and oedema. A by-product of this reaction, 4-chlorobut-2-yne-1-ol, also possessed vesicant properties.

A direct approach to 1:4-diaminobutan-2-one, from which Pyman had shown histamine could be prepared(13) was then attempted. 1:4-diaminobut-2-yne (1X; Flow Sheet B) was prepared in poor yield as described by Johnson. All attempts to hydrate this diamine with acid mercuric sulphate failed; Johnson has reported similar resistance to hydration by 1:4-bis(dimethylamino)-but-2-yne and 1:4-dipiperidinobut-2-yne(16).

In view of this the corresponding diphthalimido derivative (X; Flow Sheet B) was prepared by condensing potassium phthalimide with 1:4-dichlorobut-2 -yne in dimethyl-formamide.

Hydration of the 1:4-diphthalimidobut-2-yne was smoothly

accomplished in acetic acid solution with mercuric sulphate. Attempts to prepare the phenylhydrazone, the 2:4-dinitrophenylhydrazone and the semi-carbazone of the resulting ketone failed, a result possibly due to steric interference between the phthalimido groups and the bulky entering groups. Treatment with a reagent of smaller molecular size viz hydroxylamine gave the oxime, although even this derivative was rather unstable.

The diphthalimido ketone (XI; Flow Sheet B) was then converted to the desired diaminobutanone dihydrochloride (VII) by prolonged heating with concentrated hydrochloric acid. The diaminoketone salt was condensed with potassium thiocyanate and the resulting 2-mercaptohistamine (VIII) oxidised with ferric chloride, the histamine produced being isolated by means of picric acid. Treatment of the above picrate with hydrochloric acid furnished histamine dihydrochloride.

Attempts to desulphurise 2-mercaptohistamine by shaking or by heating under reflux with Rainey nickel in various solvents gave only starting material and a high melting amorphous solid. Oxidation with hydrogen peroxide as described by Pauly and Ludwig(18) gave no identifiable product.

The yield in every stage of the above synthesis was high and the resulting overall yield (41%) represents a considerable improvement over the earlier methods.

EXPERIMENTAL

1:4-Dichlorobut-2-yne.- This was prepared from the glycol by the action of 2.4 mols. of thionyl chloride in pyridine as described by Johnson(16), yields similar to his (83%) being obtained. In some runs a small amount of 4-chlorobut-2-yn-1-ol, b.p. 87-88°/12mm., 105-107°/25mm., n_D^{19} 1.5007, was isolated. (Found: C,45.3; H,4.9. C_4H_5OCl requires C,45.95; H, 4.8%). The phenylurethane was prepared by mixing equal volumes of 4-chlorobut-2-yn-1-ol and phenylisocyanate, the solution became warm and solidified overnight. The product crystallised from light petroleum (b.p. 60-80°) in needles m.p. 77-78°. (Found: N,6.20. $C_{11}H_{10}O_2NCl$ requires N, 6.25%)

1:4-Diphthalimidobut-2-yne (X)- (cf. Sheean and Bolhofer(17)). To a stirred solution of potassium phthalimide (31g.) in dimethylformamide (130cc.) was added slowly 1:4-dichlorobut-2-yne (10.2g.). The stirred solution was heated seven hours on a steam bath. On cooling, water (200cc.) was added and the precipitated solid (26g., 91%), m.p. 277-279°, filtered off, washed with water and dried. The diphthalimido-compound crystallised from acetic acid in pale yellow prisms, m.p. 281-282°. (Found: N,8.3. $C_{20}H_{12}O_4N_2$ requires N, 8.15%). The product was insoluble in water, ethanol,ether,dioxan, benzene, ethyl acetate, chloroform and carbon tetravhloride, moderately soluble in warm acetic acid.

1:4-Diphthalimidobutan-2-one (X1) .- To a solution of 1:4-diphthalimidobut-2-yne (3g.) in 90% acetic acid (140cc.) was added mercuric acetate (0.75g.),followed by concentrated sulphuric acid (0.5cc.) and the mixture heated under reflux for four hours. Water (200cc.) was added to the resulting solution and, after an hours standing, the precipitated solid was filtered off and washed with water. The phthalimido-ketone (2.9g., 92%) crystallised from acetic acid in plates m.p. 248-249°. (Found: C,66.3; H,4.3; N,7.5. $C_{20}H_{14}O_5N_2$ requires C,66.3; H,3.9; N7.7:).

The oxime was prepared by adding hydroxylamine hydrochloride (0.2g.) to the diphthalimido-ketone (0.2g.) in pyridine (8cc.), heating the mixture four hours on a steam bath, then heating under reflux for five minutes. When cool the mixture was poured into water where the crude oxime, precipitated as an oil, slowly solidified. One rapid crystallisation from aqueous ethanol (95%) gave the oxime (0.15g.) as fine needles m.p. 225-226°. Further crystallisation caused a drop in the melting point, probably due to hydrolysis. (Found: N.10,65. $C_{20}H_{15}O_5N_3$ requires N,11.10%).

1:4-Diaminobutan-2-one dihydrochloride (V11).- A mixture of the foregoing ketone(8.1g.), acetic acid (150cc.) and concentrated hydrochloric acid (150cc.) was heated under reflux for 36 hours, with further additions (each 40cc.) of the 1:1 acetic acid-hydrochloric acid mixture after 4 and 24 hours.

The resulting solution was treated with charcoal, filtered and evaporated to a small bulk (60cc.). On being cooled the solution deposited phthalic acid (6.1g., 82%) which was filtered off. The filtrate was evaporated under reduced pressure to 20cc., ethanol (100cc.) was then added and the solution kept at 0°. The crystalline solid thus obtained (3.15g.) was filtered off and the filtrate evaporated to obtain further small quantities of the product. The combined yield was suspended in boiling ethanol and hot water added until dissolution was effected. On being cooled the solution deposited the diamino-ketone dihydrochloride (3.25g., 83%) as elongated plates, m.p. 217° (decomp.). (Pyman$_{(13)}$ gives m.p. 221° decomp.; corr.).

2- Mercaptohistamine hydrochloride (VIII).- A solution of the diamino-ketone dihydrochloride above (3.1g.) and potassium thiocyanate (1.85g.) in water (15cc.) was evaporated to a syrup which was then heated for one hour on a water bath. Just enough hot water was then added to dissolve the inorganic salts; when kept, the solution deposited 2-mercaptohistamine (0.94g.) in th-ick needles, m.p. 244-245°. (Pyman$_{(13)}$ gives m.p. 248-249° corr.). The mother liquor was evaporated to dryness and extracted with boiling methanol (3 × 25cc.). Evaporation of the methanol in stages gave further quantities of the product (Total yield 2.84g., 90%). It proved difficult

to remove the last traces of potassium thiocyanate from the product. (Found: C,28.08; H,4.87. Calc. for $C_5H_{10}N_3S$ Cl C, 27.78; H,5.10%).

Histamine Dihydrochloride (XII).- A solution of 2-mercapto-histamine hydrochloride (155mg.) and ferric chloride hexa-hydrate (1.39g.) in water (10cc.) was heated under reflux for one hour. Enough solid sodium carbonate was then intro-duced to neutralise the mineral acid without causing pre-cipitation of the iron carbonate and a hot saturated aqueous solution of picric acid (395mg.) added. After standing for four hours the crystalline precipitate of histamine dipicrate (384mg., 80%) was filtered off. Repeated crystallisation from aqueous ethanol yielded yellow truncated needles of the monopicrate m.p. 234-235°(decomp.). (Ewins and Pyman[8] give m.p. 233-234° (decomp.)). (Found: C,35.79; H,3.06. Calc. for $C_6H_{11}O_3.C_6H_3O_7N_3$. C,35.58; H,2.99%). Treatment of a portion of the dipicrate with concentrated hydrochloric acid and benzene, followed by the removal of the benzene extract and evaporation of the aqueous layer yielded histamine hydrochloride as needles, m.p. 238-239° after crystallisation from methanol-ether. (Koessler and Hanke[7] give m.p. 244-246° corr.).

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PART 2

THE STRUCTURE OF CARPAINE

THE STRUCTURE OF CARPAINE

INDEX

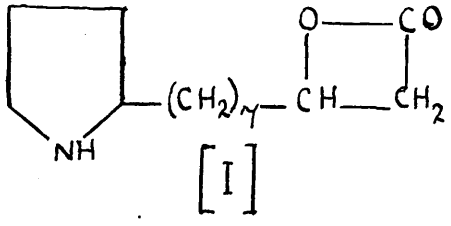
HISTORICAL.

The alkaloid carpaine was discovered and named in 1890 by Greshoff(1) who isolated it from the seeds and leaves of the pawpaw tree (Carica Papaya L.). Merck(2) assigned the formula $C_{14}H_{27}O_2N$ to the alkaloid but this was soon corrected to $C_{14}H_{25}O_2N$ by Van Rijn(3) who examined the base thoroughly in 1893. He showed carpaine to be a secondary base yielding a nitroso derivative $C_{14}H_{24}O_2N.NO$, he prepared an N-benzoyl derivative and by methylation and ethylation formed the tertiary bases from which he prepared the quaternary iodides. Further attempts by Van Rijn(4) in 1897 to elucidate the structure by hydrolysis and oxidation failed.

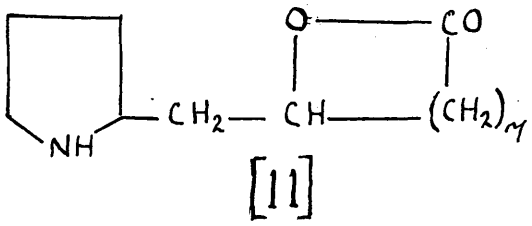
In 1910 Barger(5) demonstrated that carpaine was some type of internal anhydride. The alkaloid heated with dilute hydrochloric acid in a sealed tube gave a compound $C_{14}H_{27}O_3N$ to which he gave the name carpamic acid. He found that the ethyl ester of carpamic acid yielded a nitroso derivative hence the alkaloid was not a lactam but a lactone. Treatment of carpamic acid with potassium permanganate or nitric acid gave a compound which Barger thought to be 2:5-dimethyl-adipic acid. This led him to believe, erroniously, that carpaine contained a substituted cyclohexane ring.

Further work in 1933 by Barger, Girandet and Robinson(6) proved this oxidation product to be a mixture of azelaic and suberic acids, indicating the presence of a chain of seven

methylene groups in the alkaloid. They noted that alkaline as well as acid hydrolysis of carpaine furnished carpamic acid, further evidence for the lactone grouping. Since they failed to regenerate the lactone from the hydroxy acid it was decided that carpaine was not likely to be a γ or δ lactone. Some evidence for the presence of a pyrrolidine ring was also found. Carpaine heated with selenium at reduced pressure gave off four hydrogen atoms. Similar treatment with selenium at 15mm. gave two unidentified products both of which gave a positive pyrrole reaction with p-dimethylamino-benzaldehyde. Distillation over selenium gave yet another compound $C_{11}H_{17}N$ which gave a positive pyrrole reaction. On the above evidence they postulated structures (1) or (2) for carpaine.



[I]



[II]

However since the methylation of carpamic acid by formaldehyde gave N-methylcarpamic acid and no ketone they decided that structure (1) was more likely since Hess(7) had previously shown that pyrrolidylpropanols with secondary alcoholic groups as in structure (11) are transformed into N-methyl ketones by this treatment.

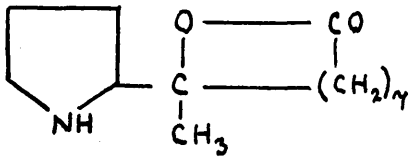
Grassman and Von Arnim(8) pointed out that the side chain was probably at the α-position since carpamic acid

failed to give the isatin colour reaction typical of pyrrolidenes with two unsubstituted α positions.

New information on the structure of carpaine appeared in 1937 when Barger, Robinson and Work(9) demonstrated the presence of a C-methyl group in carpamic acid and in its degradation products. They also demonstrated that attempted oxidation of the hydroxyl group of carpamic acid with chromic acid gave 40% of the starting material back unchanged; no ketonic product being isolated. This seems to indicate the presence of a tertiary rather than a secondary hydroxyl group as in structures (I) and (II). Attempts, however, to dehydrate carpamic acid with concentrated sulphuric acid in acetic acid failed; an unexpected result since tertiary hydroxyl groups usually dehydrate readily. Dehydration was achieved indirectly, treatment of carpamic acid with phosphorous oxychloride, basic dehydrochlorination and subsequent hydrogenation gave the crystalline deoxycarpamic acid $C_{14}H_{27}O_2N$. Continuing their investigation Barger, Robinson and Work showed that treatment of carpamic acid with red phosphorous and hydriodic acid gave a hydrocarbon $C_{14}H_{28}$, n_D^{19} 1.4325, a Kuhn-Roth estimation indicated the presence of one C-methyl group. It should be noted with reference to future structural evidence that these workers noted that myristic acid, $CH_3.(CH_2)_{12}.COOH$, on the same treatment gave a very similar hydrocarbon $C_{14}H_{28}$, n_D^{19} 1.4320. A Kuhn-Roth estimation, however, gave a negative

result.

To account for this new evidence Barger, Robinson and Work suggested structure (111) for carpaine with structure (1V) as a less likely alternative (the ethyl group being attached to the ring at an unknown point.)



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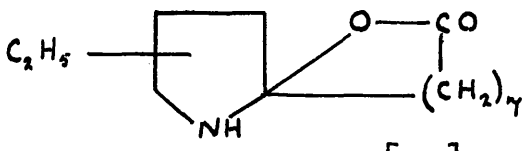
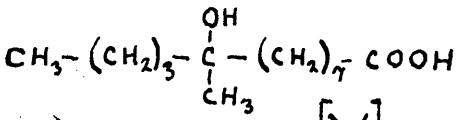


[IV]



[V]

A two-stage exhaustive methylation Hoffmann degradation procedure on carpaine, followed by hydrogenation and hydrolysis furnished an acid $C_{11}H_{28}O_3$, m.p. 20-25° which Barger, Robinson and Work believed to be 9-hydroxy-9-methyltridecoic acid (V) or 8-hydroxy-8-methyltridecoic acid, obtained from structures (111) and (1V) respectively. A Kuhn-Roth estimation on the hydroxy acid obtained indicated 0.35 C-methyl groups present. Synthesis of 8-hydroxy-8-methyltridecoic acid by Barger, Robinson and Smith(10) and comparison with the Hoffmann degradation product showed that they were not identical, the hydroxy acid was thus assumed to possess structure (V). Further synthetic work on carpaine-like compounds(11) (12) provided no conclusive evidence on the structure of carpaine.

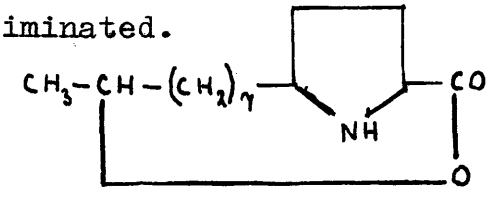
The pharmacological action of carpaine is discussed by Henry(16). An account of the use of papaya leaves by the natives

in the North Celebes is given by Fairchild(17).

One of the purposes of the present work was the synthesis of the key acid (V) and, if this structure proved to be correct for the degradation product, the extension of the method to the synthesis of carpaine itself. (Flow Sheet C; Opp. p. 18) As will be described in the following pages the synthesis of the hydroxy acid (V) has been accomplished but its properties proved to be completely at variance with those described for the carpaine degradation product.

This indication that carpaine had been inaccurately formulated was soon confirmed by the publication of Rapaport and Baldridge(13) who carried out an exhaustive methylation of carpaine using a modified technique and after hydrogenation obtained a nitrogen free acid $C_{14}H_{28}O_2$, m.p. 52-53°, which by direct comparison was shown to be myristic acid. With carpaine thus proved to have an unbranched skeleton of fourteen carbon atoms structure (111) is obviously untenable.

Rapoport and Baldridge now sought fresh evidence for the structure of carpaine(14). Carpamic acid was found to evolve no carbon dioxide with ninhydrin solution whereas proline readily evolved one mole; structures such as (V1) were thus eliminated.



(V1)

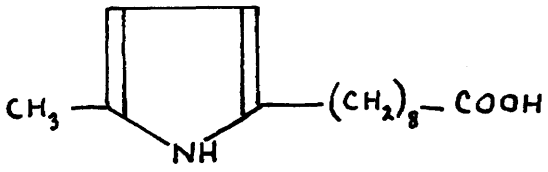
The pK of the carboxyl group of carpamic acid was found

to be 4.6. It has been shown that the pK of the carboxyl group of amino acids does not reach a value of 4.5 until the amino group is beyond the γ position[15]. This evidence combined with the isolation of azelaic acid on oxidation led Rapoport and Baldridge to the partial formulation (VII) for carpamic acid.

$$HO-\boxed{C_6H_{10}N}-(CH_2)_7-COOH \qquad (VII)$$

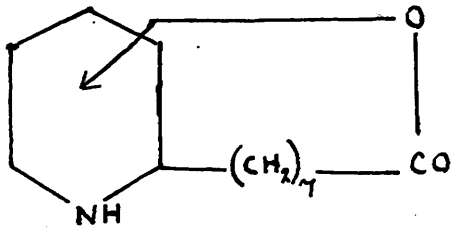
Rapoport and Baldridge now turned their attention to the nitrogen ring in carpaine[14]. They pointed out that previous conclusions about the size of the ring had been based on drastic treatment with selenium[6]. Milder dehydrogenation methods were therefor applied. Carpaine on liquid phase dehydrogenation over palladium in boiling p-cymene evolved two moles of hydrogen and one mole of water and the product was found to give a crystalline hydrochloride $C_{14}H_{21}O_2N$. HCl to which the name desoxycarpyrinic acid hydrochloride was given. The pK value for the basic group of this acid was found to be 7.0, a figure comparable to that found for α,α'-dialkylpyridines, but differing widely from the pK values for the basic groups of α,α'-dialkylpyrroles. This suggested presence of a pyridine ring was confirmed by oxidation of deoxycarpyrinic acid with potassium permanganate whereby 2:6-pyridine dicarboxylic acid was obtained. Again, the U.V. light absorption of deoxy-

carpyrinic acid closely resembled that of 2:6-lutidine but differed widely from that of the α,α'-disubstituted pyrrole (VIII)



(VIII)

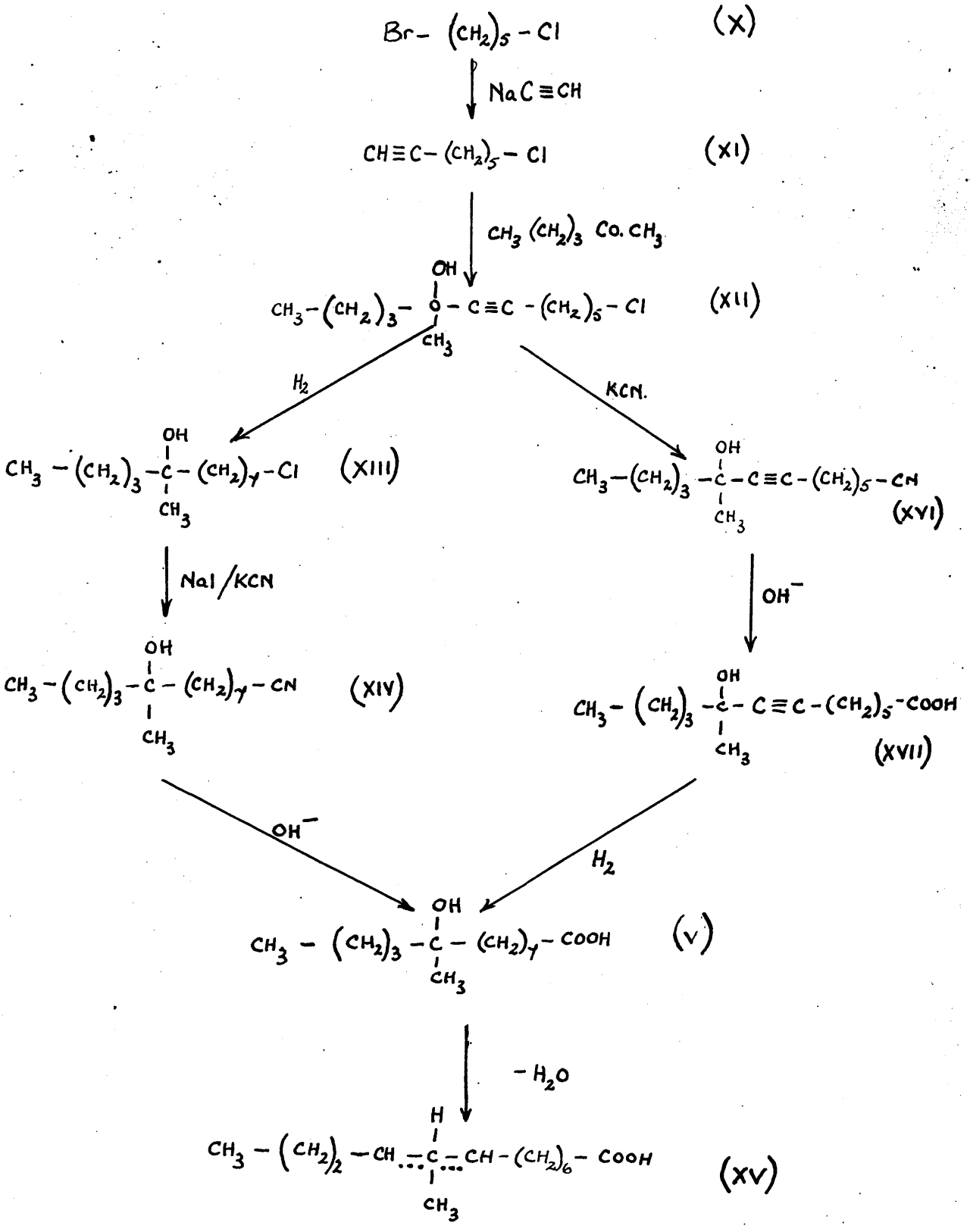
Summarising this work Rapoport and Baldridge concluded that the most likely structure for carpaine is (IX), with the



(IX)

exact point of attachment of the lactone ring to the piperidine ring still undetermined.

FLOW SHEET C


Br-(CH2)5-Cl
(X)
NaC≡CH
CH≡C-(CH2)5-Cl
(XI)
CH3(CH2)3 Co.CH3
CH3-(CH2)3-C(OH)(CH3)-C≡C-(CH2)5-Cl
(XII)
H2
KCN.
CH3-(CH2)3-C(OH)(CH3)-(CH2)7-Cl
(XIII)
CH3-(CH2)3-C(OH)(CH3)-C≡C-(CH2)5-CN
(XVI)
NaI/KCN
OH⁻
CH3-(CH2)3-C(OH)(CH3)-(CH2)7-CN
(XIV)
CH3-(CH2)3-C(OH)(CH3)-C≡C-(CH2)5-COOH
(XVII)
OH⁻
H2
CH3-(CH2)3-C(OH)(CH3)-(CH2)7-COOH
(V)
-H2O
CH3-(CH2)2-CH=C(CH3)-CH-(CH2)6-COOH
(XV)

DISCUSSION.

The following work describes the synthesis of 9-hydroxy-9-methyltridecoic acid (V) which, at the time this work was initiated, was thought to be the structure of the key degradation product of carpaine. The projected extension of the method, replacing methylbutyl ketone with  , to the synthesis of carpaine itself was abandoned when it became clear that the structure assigned to the alkaloid was inaccurate.

(See Flow Sheet C; Opposite p. 18)

1-Chlorohept-6-yne (XI) was prepared by the condensation of 1-bromo-5-chloropentane (X) with sodium acetylide in liquid ammonia. The Grignard derivative of this chloroacetylene was condensed with methylbutyl ketone in dry ether to give 1-chloro-8-hydroxy-8-methyldodec-6-yne (XII). Catalytic hydrogenation of this product furnished the fully saturated chloride (XIII) which was converted to the iodide by treatment with sodium iodide in acetone. Treatment with sodium cyanide gave the nitrile (XIV) which was not separated but immediately hydrolysed with potassium hydroxide to 9-hydroxy-9-methyltridecoic acid (V).

This acid could not be obtained in a pure state owing to very ready dehydration. Distillation gave a product which was shown by analysis to be a mixture of the hydroxy acid (V) and its dehydrated product (XV). A sharply melting S-benzyl-

isothiouronium salt prepared from this material proved to be the derivative of the unsaturated acid (XV); the exact position of the double bond undetermined. Attempts to prepare the p-phenylphenacyl derivative described by Barger[9] gave only a low melting solid, m.p.~10°, which could not be purified further.

Since it is known that tert.-∝-hydroxyacetylenes are more resistant to dehydration than saturated tert.-alcohols[18] it was decided to modify the synthesis so as to retain the triple bond until the ultimate step; as this latter step involved only a very mild catalytic hydrogenation it was felt that the procedure would provide the best chance of isolating the required hydroxyacid (V) in a pure state.

1-chloro-8-hydroxy-8-methyldodec-6-yne (XI1) was therefor converted to the nitrile via the iodide; the nitrile (XVI) was readily isolated in a pure state, no dehydration having taken place. Alkaline hydrolysis of this nitrile gave the corresponding acetylenic hydroxy acid (XVII), which again was readily purified and showed no tendency to undergo dehydration. Catalytic hydrogenation of this acid gave a product which, before being distilled, gave only a very slight positive reaction for unsaturation. After distillation, however, the product was highly unsaturated and, as before, analysis and quantitative rehydrogenation showed it to consist of a mixture of the required hydroxy acid (V) and its dehydration product (XV). The S-benzyl-isothiouronium salt obtained from this

product was identical with that obtained by the first route. Again no pure p-phenylphenacyl ester was obtainable.

It is thus certain that 9-hydroxy-9-methyltridecoic acid (V) is very unstable per se, and is very prone to dehydration even under mild conditions. This is in striking contrast to the isomeric degradation product of carpaine which emerges unscathed after treatment with an acetic acid/concentrated sulphuric acid mixture at 170° for 10 hours! This evidence contra-indicating the proposed structure for carpaine received added support almost immediately with the publication of the investigations of Rapoport already described(13) (14).

EXPERIMENTAL

Tetrahydropyran.- 2:3-Dihydropyran was hydrogenated over Rainey nickel at 100 atmospheres and 100° to give tetrahydropyran, b.p. 87-88°, n_D^{20} 1.4210. Allen and Hibbert(19) give b.p. 88-88.5°, n_D^{20} 1.4211. See also Allen and Hibbert(20).

5-Chloroamyl acetate.- This compound was prepared in 68% yield from tetrahydropyran by the action of acetyl chloride and zinc chloride as described by Synerholm(21).

1-Bromo-5-chloropentane (X).- The dihalide was prepared in 72% yield from 5-chloroamyl acetate by hydrolysis to 5-chloroamyl alcohol, followed by bromination with phosphorous tribromide as described by Newman and Wotiz(22) who give b.p. 92-93°/20mm.; n_D^{25} 1.4815 for the dihalide. Found b.p. 90-91°/18mm.; n_D^{13} 1.4864, n_D^{25} 1.4820.

1-Chlorohept-6-yne (XI).- This compound was prepared by a modified procedure of Newman and Wotiz(22).

Sodium (2g.) was added in small freshly cut slices to stirred liquid ammonia (500cc.) in a well lagged one litre 3-necked flask. Ferric nitrate (0.2g.) was then added and sodium (9.5g.) added slowly over fifteen minutes. Stirring was continued for a further fifteen minutes. Acetylene, dried by passing through concentrated sulphuric acid, was then bubbled into the stirred solution for twenty minutes. To the resulting

solution of sodium acetylide, 1-bromo-5-chloropentane (92.75g.) dissolved in an equal volume of ether, was added slowly, with stirring, over one hour. After the mixture had been stirred for a further five hours, ammonium chloride (5g.) was added. The reaction mixture was allowed to stand overnight, allowing the liquid ammonia to evaporate off. The residual liquid, after extraction with ether, washing with 1N sulphuric acid and water, was fractionated to give 1-chlorohept-6-yne (42.9g., 66%), b.p. 98-100°/80mm., n_D^{15} 1.4583. Newman and Wotiz[22] give b.p. 166°/760mm., n_D^{25} 1.4507.

Methyl-n-butyl ketone.- This ketone was prepared in 79% yield by the action of acetic anhydride on n-butylmagnesium bromide at -70° as described by Newman and Booth[23].

1-Chloro-8-hydroxy-8-methyldodec-6-yne (XII).- A 1 litre flask was fitted with a dropping funnel, a condenser(Calcium chloride tube) and a stirrer operating through a gas inlet adaptor connected to a source of dry nitrogen. Stirring was continuous throughout the preparation and air was excluded by a slow stream of nitrogen. Ethyl bromide (13.5cc.,0.166M.+5% excess) dissolved in dry ether (50cc.) was added dropwise to the dry ether (200cc.) and magnesium (4.05g., 0.166M.) in the flask. The mixture was heated for thirty minutes at 30° to complete the formation of the ethylmagnesium bromide. 1-chlorohept-6-yne (21.75g., 0.166 M.) in dry ether (50cc.) was added slowly over thirty minutes and stirring continued until no further

ethane was evolved (2 hrs.). Methylbutyl ketone (16.67g.,0.166M. in dry ether (50cc.) was then added dropwise, with cooling to keep the contents of the flask at room temperature. The mixture was then heated under reflux for 12 hours. The complex was decomposed with crushed ice and dilute sulphuric acid (200cc., 1 N.), the ether extract was washed with dilute sodium carbonate and water, dried over sodium sulphate and the solvent removed. Distillation of the residual liquid gave 1-chloro-8-hydroxy-8-methyldodec-6-yne (13.87g., 36%) as a clear liquid, b.p. 131-134°/3mm., n_D^{17} 1.4776. (Found: C,67.01; H,9.86. $C_{13}H_{23}OCl$ requires C,67.65; H,10.05%).

The corresponding S-thiourea picrate was prepared as follows. 1-chloro-8-hydroxy-8-methyldodec-6-yne (0.2g.) was heated under reflux for 18 hours with potassium iodide (0.33g.) in ethanol (5cc.). The mixture was reduced in volume to 2cc., then cooled and filtered. Thiourea (0.1g.) was added and the mixture heated under reflux for 30 minutes. Picric acid (0.1g.) dissolved in the minimum volume of hot ethanol was then added, resulting in the immediate precipitate of the S-th-iourea picrate (0.3g.) which crystallised from nitroethane as yellow needles decomposing explosively at 328°. (Found : N,14.62. $C_{20}H_{29}O_8N_5S$ requires N,14.02%).

1-Chloro-8-hydroxy-8-methyldodecane (XIII).- 1-Chloro-8-hydroxydodec-6-yne (8.01g.) was hydrogenated in ethyl acetate (100cc.) over Adams' catalyst (0.4g.). The theoretical volume

of hydrogen (1564cc.; 13°/762mm.) was absorbed in 3½ hours, after which time the uptake of hydrogen had practically ceased. After removal of catalyst and solvent the product was fractionated to give 1-chloro-8-hydroxy-8-methyldodecane (6.04g., 75%) as a clear liquid, turning yellow on standing, b.p. 142-145°/23mm., n_D^{20} 1.4522, n_D^{24} 1.4504. (Found : C,66.46; H,10.89. $C_{13}H_{27}OCl$ requires C,66.49; H,11.60%). On being tested with bromine in carbon tetrachloride no appreciable unsaturation was found in the product.

9-Hydroxy-9-methyltridecoic acid (V).- 1-Chloro-8-hydroxy-8-methyldodecane (6.04g.) was heated under reflux for 26 hours with sodium iodide (7.7g.) in dry acetone (40cc.). The solution was cooled and filtered. After removal of the acetone *in vacuo* potassium cyanide (4g.) in 80% aqueous ethanol (70cc.) was added and the mixture heated under reflux for 33 hours. After the addition of potassium hydroxide (4g.) in water (5cc.) the heating was resumed for a further 14 hours. The bulk of the ethanol was removed under reduced pressure, the residue diluted with water and the mixture extracted with ether to remove non-acidic material. After acidification with dilute sulphuric acid the aqueous layer was reextracted with ether, the extract dried over sodium sulphate and after removal of the solvent the residue was fractionated at 0.15mm..
The product boiled over a range 118-132° with some decomposition, the colours of the fractions ranging from brown to

yellow. Some solid crystallised out at room temperature, all fractions were completely solid at 0°. The fractions were recombined and triturated with petrol (b.p. 60-80°); a small amount of white acidic solid remaining undissolved was crystallised from carbon tetrachloride to give needles m.p. 101° (Found : C,52.22; H,7.42. Calc. for $C_7H_{12}O_4$ C,52.50; H,7.55%). This by-product was probably pimelic acid, HOOC-$(CH_2)_5$-COOH (lit. m.p. 103°).

Refractionation of the petrol extract gave five new fractions, b.p. 114-126°/0.08mm., with refractive indices ranging from n_D^{17} 1.4534 to 1.4547. This time all fractions remained liquid at -16°, adsorbed bromine strongly and immediately decolourised aqueous potassium permanganate. Analysis of a centre cut gave the following figures (Found : C, 72.35; H, 11.32. $C_{14}H_{28}O_3$ requires C, 68.83 H, 11.55%. $C_{14}H_{26}O_2$requires C, 74.31; H, 11.58%). Analysis of other fractions gave similar results, all indicating that dehydration had taken place to a considerable extent.

An S-benzyl isothiouronium salt of the product, prepared in the normal manner, crystallised as plates, m.p. 138°, from aqueous ethanol. Analysis proved it to be the salt of the dehydrated acid (XV). (Found : C. 67.16; H, 9.13, N, 7.09. $C_{14}H_{28}O_3.C_8H_{10}N_2S$ requires C, 64.40; H, 8.78; N, 6.84%. $C_{14}H_{26}O_2.C_8H_{10}N_2S$ requires C, 67.30; H, 9.24; N,7.14 %)

Attempts to prepare the p-phenylphenacyl ester of the product gave only an impure derivative,m.p. 8-12°, which defied

further purification.

1-Cyano-8-hydroxy-8-methyldodec-6-yne (XVI).- 1-Chloro-8-hydroxy-8-methyldodec-6-yne (6.27g.) was heated under reflux for 20 hours with sodium iodide (9g.) in dry acetone (50cc.). The reaction mixture was reduced in volume to 15cc., cooled and filtered. After removal of the acetone from the filtrate, sodium cyanide (5.5g.) in 80% aqueous ethanol (100cc.) was added and the mixture heated under reflux for 46 hours. After filtration the ethanol was removed, the residue diluted with water and the mixture extracted with ether. After washing the ether extract with water and drying over sodium sulphate the product was fractionated to give 1-cyano-8-hydroxy-8-methyldodec-6-yne (3.56g., 60%) as a clear liquid, b.p. 185-187°/24mm., n_D^{14} 1.4730. (Found : C, 75.75; H, 10.00; N, 6.02. $C_{14}H_{23}O N$ requires C, 75.97; H, 10.47; N, 6.33%).

1-Carboxy-8-hydroxy-8-methyldodec-6-yne (XVII).- 1-Cyano-8-hydroxy-8-methyldodec-6-yne (3.56g.) was heated under reflux for 16 hours with sodium hydroxide (5g.) in 80% aqueous ethanol (100cc.). The solution was diluted with water and extracted with ether to remove non-acidic materials. The aqueous layer was then acidified with dilute sulphuric acid and extracted with ether. The ethereal extract was washed repeatedly with water until completely free from mineral acid

(in another run a trace of sulphuric acid was found to cause appreciable decomposition during the following fractionation). After drying the ethereal extract over sodium sulphate and removal of the solvent the crude product was fractionated to furnish 1-carboxy-8-hydroxy-8-methyldodec-6-yne (2.24g., 60%) as a clear liquid, b.p. 134-136°/0.08mm., n_D^{18} 1.4806. (Found : C, 70.10; H, 9.85. $C_{14}H_{24}O_3$ requires C, 69.96; H, 10.06%).

The corresponding S-benzyl isothiouronium salt crystallised from aqueous ethanol as plates m.p. 127°. (Found : N, 6.97. $C_{14}H_{24}O_3.C_8H_{10}N_2S$ requires N, 6,89%).

9-Hydroxy-9-methyltridecoic acid (V).- The above acetylenic acid (2.24g.) was hydrogenated over Adams' catalyst (0.1g.) in methyl acetate (50cc.). When 665cc. of hydrogen had been absorbed (70 mins.) the uptake of hydrogen ceased (theor. 640cc; 15°/762mm.). Removal of catalyst and solvent gave a colourless liquid which gave only a faint positive response to the usual unsaturation tests. This product distilled over the range 127-137°/0.1mm., n_D^{15} 1.4451 - 1.4491, the main bulk distilling at 133-135°/0.1 mm., n_D^{15} 1.4488. All fractions now absorbed bromine with avidity and decolourised aqueous potassium permangate immediately in the cold. Analysis of the middle cut (n_D^{15} 1.4488) showed the product to be a mixture of the required hydroxy acid (V) and its dehydrated product (XV). (Found : C, 72.54; H, 11.10. $C_{14}H_{28}O_3$ requires

C, 68.83; H, 11.55. $C_{14}H_{26}O_3$ requires C, 74.28; H,11.58%). Other fractions gave similar results. This was confirmed by hydrogenation of the distilled product, 1.246g. of which rapidly absorbed a further 64 cc. of hydrogen at 15°/756 mm.. (Theor. if fully unsaturated :- 173 cc.). This indicates that the dehydrated product was present to the extent of 40%.

The S-benzyl isothiouronium salt prepared from the distilled product crystallised from aqueous ethanol as plates, m.p. 138°, undepressed on admixture with the derivative prepared via the first route.

Attempts to prepare the p-phenylphenacyl ester gave a yellow oil, which on chromatography (benzene-alumina) gave three bands. The first band eluted was starting material, p-phenylphenacyl chloride. The remaining two bands gave oils which though solid at 0° could not be crystallised to give a pure product.

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PART 3

SYNTHETIC STUDIES ON BAIKIAIN

SYNTHETIC STUDIES ON BAIKIAIN

INDEX

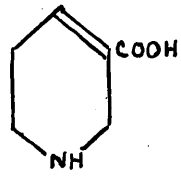
HISTORICAL

During an investigation in 1950 of the constituents of timbers known to be resistant to fungal decay and the attack of wood destroying insects King, King and Warwick[1] examined the heartwood of Rhodesian Teak (Baikiaea Plurijuga). They isolated from the timber a high melting nitrogenous solid, soluble in methanol and water and insoluble in other organic solvents.

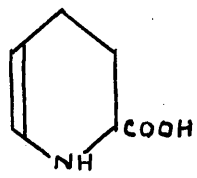
Analysis of the compound, which they named "baikiain", and its derivatives gave the emperical formula $C_6H_9O_2N$. The high melting point of baikiain and its solubility relationships pointed strongly to an amino acid structure. The absence of a $-CH(NH_2)-COOH$ group was shown by the ninhydrin test; no purple colour was produced; but the yellow-brown colouration obtained was typical of cyclic α-amino acids, e.g. proline.

On catalytic hydrogenation one mole of hydrogen was absorbed, suggesting that baikiain was a methylpyrroline or a tetrahydropyridine carboxylic acid. The rotational value of dihydrobaikiain was observed to resemble closely the value found for L-α-pipecolinic acid[2]. The nature of the ring and the position of the carboxyl group in baikiain was shown by a zinc dust distillation when the product obtained proved

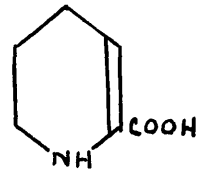
to be ∝-picoline; a similar reduction of the carboxy group was obtained by the action of zinc dust on guvacine (1) producing β-picoline(3). This evidence led King et al to consider structures (11)-(V) for baikiain.



(1)



(11)



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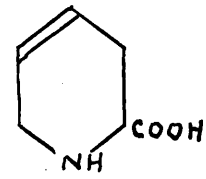
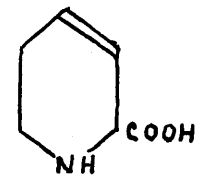


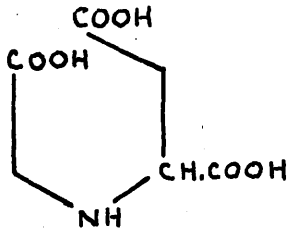
(1V)



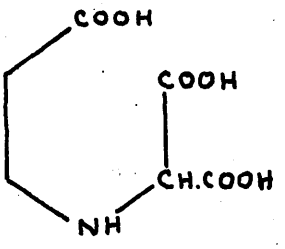
(V)

They dismissed the possibility of a Δ^1 or Δ^6 structure on the grounds that the extreme stability of baikiain to concentrated acids is incompatable with a Schiffs base structure. Again the stability of baikiain to acids and alkalis eliminated structure (11) with its relatively unstable vinylamine group. Structure (111) was discarded because of the absence of an asymmetric carbon atom. Of the two remaining structures (1V) seemed to be more likely since the 3,4-unsaturated acid (V) would be expected to undergo ready racemisation via the tautomeric 2,3-unsaturated structure. However, baikiain on heating for twelve hours with 40% aqueous sodium hydroxide was only partially racemised.

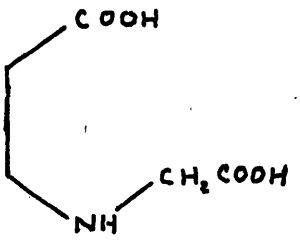
In order to confirm the position of the double bond King et al(1) oxidised baikiain with ozone and hydrogen peroxide ; the product was esterified and the resulting trimethyl ester characterised as a picrate and a picronolate.



(V1)



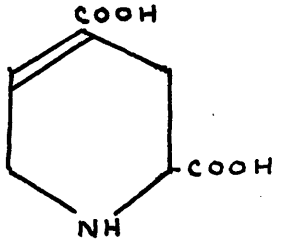
(V11)



(V111)

The optically active form of (V1), the expected oxidation product of (1V), and of (V11) and (V111), the expected oxidation forms of (V) were prepared synthetically. Comparison of these compounds and their derivatives with the oxidation product showed the latter to be identical with (V1), thus indicating that baikiain possessed structure (1V).

An attempted synthesis of baikiain from L-glutamic acid did not lead to the isolation of the synthetic amino acid. The penultimate stage L-1:2:3:6-tetrahydropyridine-2:4-dicarboxylic acid (1X) was isolated but proved resistant



(1X)

to decarboxylation. However, heating (1X) in a sealed tube with concentrated hydrochloric and sublimation of the resulting product gave an amino acid, contaminated with ammonium chloride, which gave a yellow-brown colouration with ninhydrin solution unlike the orange colour produced by (1X). Paper chromatography of this crude product and subsequent

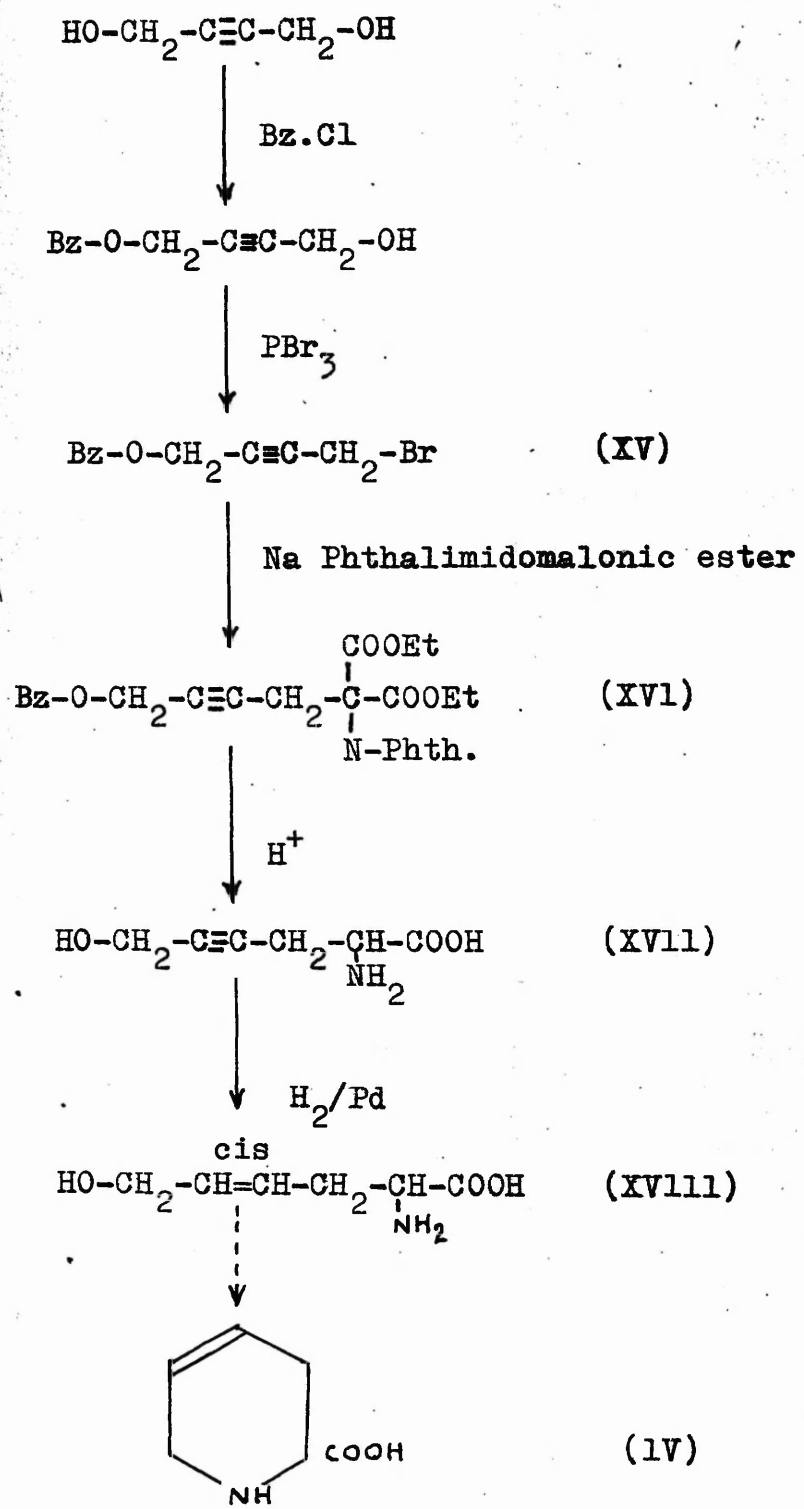
development with ninhydrin gave a spot identical in position and in appearance with that given by baikiain hydrochloride run as a standard on the same paper.

Baikiain has also been isolated at the "Forest Products Research Laboratory"(4), during an investigation of the resistance of Rhodesian Teak to fungal decay; it was found to have no fungicidal action in agar media. In a later report from the same source Carruthers and Farmer(5) reported differences in the physical properties, especially the optical rotations, of baikiain isolated by them and of baikiain isolated by King et al. Believing these discrepances to be caused by partial racemisation either in the wood or during extraction they are attempting the resolution of a completely racemised sample of baikiain via the D-camphor-10-sulphonic acid salt. The rotational values of the completely resolved baikiain should indicate the degree of racemisation in the isolated baikiain.

Further notes on improved extraction techniques for baikiain and on observed rotational values have been published by King(6) and by Carruthers and Farmer(7).

King and King(6) have pointed out that the catalytic reduction of baikiain affords a very convenient source of L-pipecolinic acid which has been found to be widely distributed in leguminous plants(8).

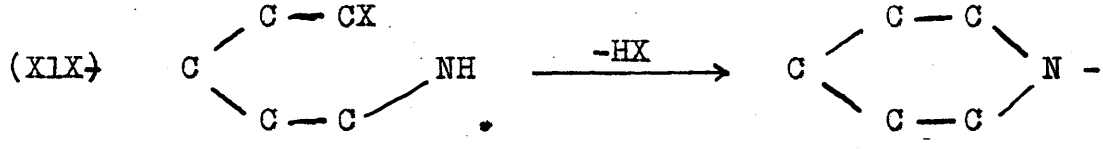
FLOW SHEET E


HO-CH2-C≡C-CH2-OH
Bz.Cl
Bz-O-CH2-C≡C-CH2-OH
PBr3
Bz-O-CH2-C≡C-CH2-Br
(XV)
Na Phthalimidomalonic ester
COOEt
Bz-O-CH2-C≡C-CH2-C-COOEt
N-Phth.
(XVl)
H+
HO-CH2-C≡C-CH2-CH-COOH
NH2
(XVll)
H2/Pd
cis
HO-CH2-CH=CH-CH2-CH-COOH
NH2
(XVlll)
COOH
NH
(lV)

DISCUSSION

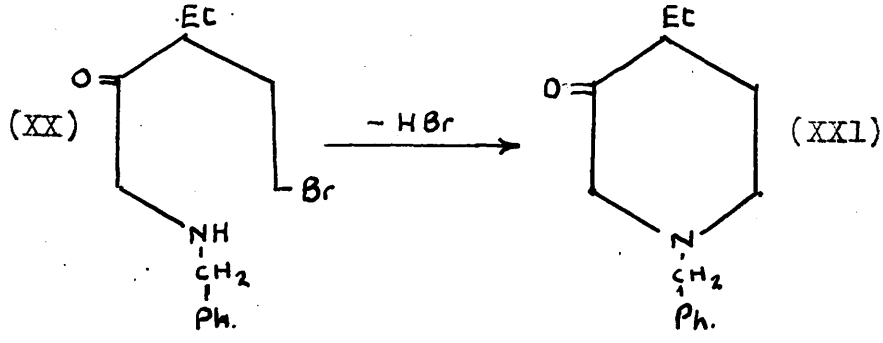
Since King, King and Warwick(1) did not succeed in isolating baikiain a further attempt at synthesis seemed desirable in order to provide unequivocal evidence for the structure of the amino acid.

For the preparation of piperidine and its derivatives three general methods have been used in effecting ring closure at the nitrogen atom(9). Starting with a compound of type (XlX)



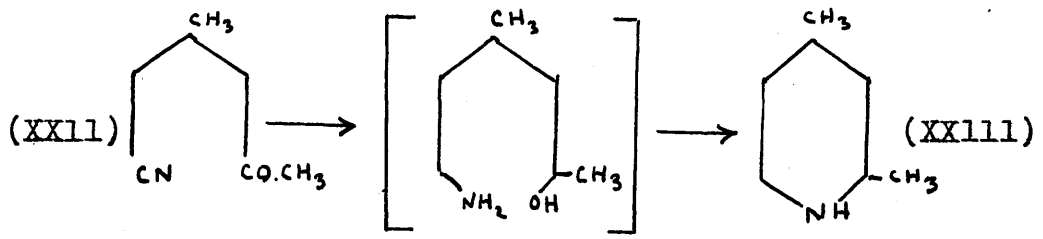
(a) X=NH_2. Ladenburg(10) prepared piperidine by the action of heat on pentamethylenediamine hydrochloride.

(b) X=Halogen Work(13) prepared the substituted piperidone (XXl) by the action of sodium carbonate on the bromide (XX)



This method also embraces syntheses starting with 1:5-dihalides since the closure method involves the heating of this type of compound with ammonia or amines, with the intermediate formation of an 1:5-halogenoamine(11) (12).

(c) X=OH It was originally thought that 1:5-amino-alcohols were incapable of existence in a free state but immediately underwent ring closure to the corresponding piperidene. Wohl and Maag(14) have shown that the keto nitrile (XXII) on catalytic reduction gives the substituted piperidene (XXIII) directly.



Similar results were obtained by other workers(15). 1-Amino-5-pentanol, however, has been recently prepared from dihydropyran(16) and was shown to be stable to distillation.

The use of Method (a) was envisaged in the first attempt to synthesise baikiain since it was possible to obtain the required intermediate for cyclisation 1:5-diamino-3(cis)-ene-1-carboxylic acid (XIII) by a five-stage synthesis from the readily available but-2-yn-1:4-diol.

(See Flow Sheet D; Opposite p.34)

1:4-dichlorobut-2-yne was prepared by the action of thionyl chloride on but-2-yn-1:4-diol as described by Johnson(17). The dihalide was then condensed with one mole of potassium phthalimide in dimethylformamide to give 1-phthalimido-4-chlorobut-2-yne (X). This latter compound was then condensed with the sodium salt of N-formamidomalonic ester in dry ethanol and the condensation product (X1) catalytically semi-hydrogenated to yield 1-formamido-1:1-dicarbethoxy-5-phthalimidopent-3(cis)-ene (X11). The cis configuration of the double bond produced was assigned on the basis of many precedents(18a). Hydrolysis of (X11) with hydrochloric acid gave the exceedingly hygroscopic dihydrochloride of the amino acid (X111), but sulphuric acid hydrolysis gave the beautifully crystalline sulphate; the product was further characterised as its N:N'-dibenzoyl derivative.

To confirm the structure of (X111) the sulphate was completely hydrogenated to the sulphate of D,L-lysine which was characterised as its N:N-dibenzoyl derivative.

In order to allay doubts concerning the resistance of the cis-double bond in (X111) to stereomutation during the acid hydrolysis, the synthesis was modified so as to effect the semi-hydrogenation after the hydrolysis. Heating the acetylenic intermediate (X1) with hydrochloric acid gave the crystalline dihydrochloride of 1:5-diamino-1-carboxypent-3-yne (X1V) Catalytic semi-hydrogenation of (X1V) gave a mixture

of the hygroscopic dihydrochloride of (XIII) already encountered and a crystalline non-hygroscopic monohydrochloride; both hydrochlorides gave the same N:N'-dibenzoyl derivative already obtained from the product of the first route. It thus seems clear that the cis configuration of the double bond in (XIII) is not affected by the comparatively drastic acid treatment.

All attempts at ring closure to baikiain on the mono and dihydrochlorides and on the sulphate of the amino acid (XIII) failed. The action of heat and of acids and alkalis under various conditions gave either the starting material back unchanged or caused decomposition.

This approach was accordingly abandoned in favour of a procedure involving Method (c), i.e. the ring closure of a 1:5-amino alcohol. The attractiveness of this method was further enhanced by the fact that Pleninger[19] had already effected a very similar ring closure involving the synthesis of proline by heating the amino acid (XXIV) with concentrated

$$HO-(CH_2)_3-CH(NH_2)-COOH \qquad (XXIV)$$

hydrochloric acid. It seemed likely, therefore, that the application of this procedure to (XVIII) (See Flow Sheet E ; Opposite p. 34) would lead to baikiain.

$$HO-CH_2-\overset{cis}{CH=CH}-CH_2-\underset{NH_2}{CH}-COOH \qquad (XVIII)$$

Synthesis of this key intermediate started from the benzoate of 4-bromobut-2-yn-1-ol (XV) the preparation of which, from but-2-yn-1:4-diol, is described in a later section (p. 73).

Attempted condensations of 1-bromobut-2-yn-1-ol benzoate with the sodium salt of formamidomalonic ester in dry ethanol and with the sodium salt of acetamidomalonic ester in dry toluene failed; no condensation products could be isolated, and in the first case approximately 85% of the theoretical amount of ethyl benzoate was recovered. The sodium salt of phthalimidomalonic ester, prepared by the addition of phthalimidomalonic ester to atomised sodium in toluene, was heated with the bromobenzoate (XV), but the bulk of the bromobenzoate (75%) was recovered unchanged. In this experiment it was noted that this method of obtaining the sodium salt of phthalimidomalonic ester yielded the derivative as a lumpy aggregate, obviously not the optimum physical state for further reaction. In order to avoid this difficulty the sodium salt of phthalimidomalonic ester was prepared separately as described by Barger and Weichselbaum[20], ground into a fine powder and heated under reflux for three days with the bromobenzoate in toluene ; a 70% yield of the condensation product, the benzoate of 1-phthalimido-1:1-dicarbethoxypent-3-yn-5-ol (XVl), was thus obtained.

This condensation product on hydrolysis gave the hydroxyamino acid, 1-amino-1-carboxypent-3-yn-5-ol (XVll). Catalytic

semi-hydrogenation over palladium-charcoal gave the corresponding cis-ethylenic amino acid (XVlll). A structural check was provided at this point by the complete hydrogenation of this product to the known 6-hydroxy-2-aminocaproic acid.

All ring closure attempts on the hydroxy amino acid (XVlll) and its derivatives failed. The action of heat, acids and alkalis gave the starting material back unchanged or caused decomposition. The hydroxy amino acid was particularly sensitive to the action of warm dilute alkalis; decomposition taking place, yielding an oil with a strong piperidine-like odour.

It was suspected that the presence of the free carboxyl group might be the cause of this resistance to cyclisation, owing to zwitterion formation. To eliminate this factor cyclisation was attempted on the corresponding methyl ester and amide, but again no ring-closed product could be isolated.

It was interesting that all the above unsaturated amino acids gave only a yellow-brown colour with aqueous ninhydrin while the corresponding saturated compounds readily gave the expected purple colour. With citrate-buffered ninhydrin solution, however, all the amino acids behaved normally. The results are summarised in Table 1, page 52.

EXPERIMENTAL PART 1

1:4-Dichlorobut-2-yne.- This was prepared from the glycol by the action of 2.4 moles of thionyl chloride in pyridine as described by Johnson[17].

1-Phthalimido-4-chlorobut-2-yne (X).- (cf. Sheehan and Bolhofer[18]). A solution of 1:4-dichlorobut-2-yne (57g.) in dimethylformamide (350 cc.) was heated to 95° on a water bath. Potassium phthalimide (88g.) was added, with stirring, over a period of two hours. Heating and stirring were continued for a further eight hours. Water (1500cc.) was added to the cooled mixture and the filtered product washed with more water (500cc.) The crude product (160g.) was heated under reflux with glacial acetic acid (300cc.) and filtered hot. Crude 1:4-diphthalimido-but-2-yne (13g.), m.p. 278-279°, remained undissolved. (Fraser and Raphael[21] give m.p. 281-282°). A further quantity of 1:4-diphthalimidobut-2-yne (14.8g.) crystallised out on cooling. Water (1000cc.) was added to the mother liquor and the resulting crude 1-phthalimido-4-chlorobut-2-yne filtered off and recrystallised from ethanol from which solvent it formed pale yellow needles, m.p. 116-117°, (44g., 41%). An analytical sample had m.p. 118-119°. (Found : C, 62.0; H, 3.4. $C_{12}H_8O_2NCl$ requires C, 61.7; H, 3.4%)

1-Formamido-1:1-dicarbethoxy-5-phthalimidopent-3-yne (XI).- Ethyl formamidomalonate (16.24g.) was added to dry ethanol (300cc.) in which sodium (1.84g.) had been dissolved. The solution was heated at 50° for one hour. 1-Phthalimido-4-chlorobut-2-yne (18.68g.) was then added and the stirred mixture heated under reflux for 14 hours. The volume was reduced to 100cc. and acetic acid (1cc.) added. On the addition of water the crude product was precipitated as a viscous oil which rapidly solidified. Crystallisation from light petrol (b.p. 60-80°) containing ethanol (5%) furnished the condensation product as needles, m.p. 122°, (19.2g., 60%). (Found: C, 60.1; H, 4.8. $C_{20}H_{20}O_7N_2$ requires C, 60.0; H, 5.0%)

1-Formamido-1:1-dicarbethoxy-5-phthalimidopent-3(cis)-ene (XII).- The above condensation product (XI), (7.1g.), was catalytically semi-hydrogenated in ethyl acetate (200cc.) over 10% palladium-charcoal (0.4g.). After absorbing 452cc. of hydrogen the uptake of hydrogen ceased (theoretical volume 468cc., 19°/763mm.). Removal of catalyst and solvent followed by crystallisation from petrol (60-80°) containing ethanol (5%) gave the product as needles, (6.8g., 95%), m.p. 110°. (Found; C, 59.5; H, 5.3. $C_{20}H_{22}O_7N_2$ requires C, 59.4; H, 5.5%).

1:5-Diamino-1-carboxy~~hept~~pent-3(cis)-ene sulphate (XIII).- The above product (XII) (6.4g.) was heated under reflux for five hours with dilute sulphuric acid (12cc. conc. acid + 100cc. water). The volume was reduced to 20cc. and the solution cooled to 0°. After removal of the phthalic acid by filtration, ethanol (300cc.) was added and the mixture allowed to stand overnight at 0°. The filtered product was recrystallised by dissolving the crude material in a small volume of hot water and adding boiling ethanol till the solution became turbid. On being cooled the solution deposited the sulphate as feathery needles, (1.56g., 41%), m.p.234-235°. The main loss of product occurred during the purification. (Found : C, 29.8; H, 6.1; N,11.7. $C_6H_{12}O_2N_2.H_2SO_4$ requires C, 29.8; H, 5.8; N, 11.6%).

The N:N'-dibenzoyl derivative prepared by the usual Schotten-Baumann procedure crystallised from water as prisms, m.p. 179°. (Found : N, 8.1. $C_{20}H_{20}O_4N_2$ requires N, 7.95%)

Hydrolysis of (XIII) with 16 N. hydrochloric acid gave the dihydrochloride of the diamino acid as an extremely hygroscopic solid. The N:N'-dibenzoyl derivative was identical to that prepared from the diamino acid sulphate. Treatment of the dihydrochloride with ammonia followed by sulphuric acid gave the diamino acid sulphate, m.p. 234-235°, identical with that produced by direct hydrolysis with sulphuric acid.

N:N'-Dibenzoyl-D,L-lysine.- 1:5-Diamino-1-carboxypent-3-ene (0.22g.) dissolved in water (10cc.) was fully hydrogenated over palladium-charcoal catalyst (0.05g.). After absorbsion of 28cc. hydrogen the uptake of hydrogen ceased (theor. vol. 22.5cc., 18°/759mm.). The corresponding N:N'-dibenzoyl derivative made by the Schotten- Baumann technique crystallised from aqueous acetone (10%) as needles, m.p. 142-143°. (Von Braun[22] gives m.p. 145-146°) (Found : C, 67.3; H, 6.11; N, 7.73. Calc. for $C_{20}H_{22}O_4N_2$ C,67.8; H, 6.25; N, 7.90%)

1:5-Diamino-1-carboxybut-3-yne dihydrochloride (XlV).- 1-Formamido-1:1-dicarbethoxy-5-phthalimidopent-3-yne (15g.) was heated under reflux for six hours with hydrochloric acid (6N., 120cc.). The volume was reduced to 30cc. and the phthalic acid filtered off. After reducing the volume in vacuo to 10cc. the crude amino acid hydrochloride was precipitated with acetone. Recrystallisation from aqueous ethanol (90%) gave the diamino acid dihydrochloride as needles, (6.12g., 76%), m.p. 192-194° (decomp.), softening taking place over the range 120-140°. (Found : C, 33.7; H, 5.65; N, 13.06. $C_6H_{10}O_2N_2.2HCl$ requires C, 33.7; H, 5.84; N,13.03%)

The N:N'-dibenzoyl derivative prepared by the Schotten-Baumann method crystallised from aqueous ethanol (10%) as prisms, m.p. 176°. (Found: N,8.2. $C_{20}H_{18}O_4N_2$ requires N,8.0%)

1:5-Diamino-1-carboxy~~but~~pent-3(cis)-ene hydrochloride(XIII).- 1:5-Diamino-1-carboxy~~but~~pent-3-yne dihydrochloride (3.93g.) was dissolved in water (40cc.) and catalytically semi-hydrogenated over palladium-charcoal (0.3g., 10%), the process taking 3.5 hours (443cc. hydrogen, 20°/754mm.). After removal of the catalyst the volume was reduced in vacuo to 5cc. and acetone (100cc.) added. The precipitated oil solidified on heating with ethanol. Recrystallisation from aqueous ethanol (95%) gave the diamino acid hydrochloride as prisms (1.57g., 53%), m.p. 257-258° (decomp.). (Found: C, 39.9; H, 6.94; N, 15.6. $C_6H_{12}O_2N_2$.HCl requires C, 39.9; H, 7.25; N, 15.5%).

The diamino acid hydrochloride was exceedingly soluble in water, soluble in warm methanol and sparingly soluble in boiling ethanol. It was insoluble in other organic solvents. The N:N'-dibenzoyl derivative, m.p. 179°, was identical to that obtained from the diamino acid sulphate described above. On addition of acetone to the ethanolic mother liquors, after removal of the hydrochloride, approximately 1g. of the hygroscopic dihydrochloride precipitated out. Benzoylation gave an N:N'-dibenzoyl derivative, m.p. 179°, identical with that obtained from the hydrochloride. As a further check treatment of the diamino acid hydrochloride with ammonia and sulphuric acid furnished a diamino acid sulphate, m.p. 234-235° (decomp.), identical with that formed by the sulphuric acid hydrolysis of 1-formamido-1:1-dicarbethoxy-5-phthalimido-~~but~~pent-3-yne (XII) previously described.

SUMMARY OF RING CLOSURE ATTEMPTS

(a) On 1:5-diamino-1-carboxy~~hex~~pent-3(cis)-ene hydrochloride.

1. Sublimed at $240^{\circ}/5\times10^{-5}$mm. unchanged.
2. Heated under reflux in water for 14 hours; starting material isolated as the N:N'-dibenzoyl derivative.
3. Heated under reflux for 10 minutes, and in a repeat experiment for 60 minutes, with 2N sodium carbonate. Starting material only was recovered as the N:N'-dibenzoyl derivative.
4. Heated with 1N sodium hydroxide solution (2.2 mols.). Decomposition took place, a piperidine-like odour was noticed. No benzoyl derivative could be isolated.
5. Dissolved in water and heated in a Carius tube. Heating at 200° and 250° for six hours gave a small amount of oil with a piperidine-like odour. A small amount of starting material was recovered unchanged. Heating at 180° for twelve hours gave a 50% recovery of starting material plus a small amount of brown oil.
6. Heated under reflux for twelve hours with concentrated hydrochloric acid; product recovered unchanged.
7. Heated in a dry tube at its decomposition point for two minutes. No ring closed product recovered.
8. Slow addition of one mole of sodium nitrite to the amino acid hydrochloride dissolved in water cooled to 5°. No benzoyl derivative could be isolated from

the reaction mixture.

(b) <u>On the diamino acid sulphate</u>.

1. Heating with 0.3N barium hydroxide caused decomposition
2. " " o.1N sodium " " "
3. Heating at the melting point caused decomposition.
4. Isolation of the sodium salt was achieved by the addition of three equivalents of N sodium hydroxide followed by evaporation to dryness. Heating this salt alone caused decomposition; heating the salt in aqueous solution left the compound unchanged.

(c) <u>On the amino acid dihydrochloride</u>.

1. Boiling in toluene for eight hours, removing any water formed by azeotropic distillation, gave only the hygroscopic starting material.
2. Repeated fusion merely caused decomposition.

EXPERIMENTAL PART 2

4-Bromobut-2-yn-1-ol benzoate (XV).- This compound was prepared from but-2-yn-1:4-diol by treatment of the corresponding monobenzoate with phosphorous tribromide.(For experimental details see page 73)

1-Phthalimido-1:1-dicarbethoxypent-3-yn-5-ol benzoate (XVI).- Sodium (2.22g.) was dissolved in dry ethanol (200cc.); ethyl phthalimidomalonate (29.4g.), prepared as described by Osterberg(26), was then added and the mixture heated for one hour at 50°. The ethanol was removed in vacuo and the ethanol of crystallisation removed by heating the residual solid for one hour at 150°/0.5mm.. The sodium salt thus formed was finely powdered and suspended in dry toluene (500cc.). 4-Bromobut-2-yn-1-ol benzoate (20.36g.) was added and the mixture heated under reflux for 65 hours; calcium chloride tubes being used to prevent ingress of moisture. After filtering hot through a layer of animal charcoal in a sintered glass funnel, acetic acid (2cc.) was added. When cool the toluene solution was shaken with dilute sulphuric acid (1N., 2×100cc.), water, dilute sodium bicarbonate and water. After drying the toluene layer over sodium sulphate the solvent was removed in vacuo, the crude semi-solid product was dissolved in ethanol (40cc.) and cooled to -16°. The condensation product (XVI) , (26.54g., 69%) crystallised

out as elongated plates, m.p. 61-63°. An analytical sample recrystallised from n-butyl ether as plates, m.p. 63-64°. (Found : C, 65.2; H, 4.7. $C_{26}H_{23}O_8N$ requires C, 65.4; H,4.8%)

1-Amino-1-carboxypent-3-yn-5-ol (XVII).- The above benzoate (XVI), (6.7g.), was dissolved in a mixture of ethanol(50cc.) and hydrochloric acid (12N., 10cc.). The mixture was heated under reflux for nine hours, further additions of 12N hydrochloric acid (10×10cc.) being made at 30 minute intervals. The reaction mixture was then reduced in volume to 30cc., cooled, and the mixture of benzoic and phthalic acids thus precipitated, filtered off. The filtrate was reduced in volume to 5cc. in vacuo. Acetone (150cc.)was then added, precipitating the hygroscopic hydroxy amino acid hydrochloride. As much hydrochloric acid as possible was removed by repeatedly triturating the semi-solid product with acetone. The crude hydroxy amino acid hydrochloride was then dissolved in water (20cc.) and anion exchange resin "Amberlite IR-4B" (3g.) added. The mixture was shaken vigourously for four hours and the resin filtered off. Removal of the water under reduced pressure gave the crude hydroxy amino acid contaminated with glycine. The glycine was conveniently removed by sublimation at $180^{\circ}/5\times10^{-5}$mm. Recrystallisation of the residue from methanol furnished the hydroxy amino acid (XVII) (1.5gms; 75%) as needles m.p. 192° (decomp.). (Found; C. 50.0; H, 6.5; N, 9.3. $C_6H_9O_3N$ requires C, 50.3; H, 6.3; N, 9.7%)

Quantitative microhydrogenation showed the presence of 2.2 double bonds.

Repeated attempts to prepare the benzoyl derivative by the Schotten-Baumann technique gave no isolatable product.

The 3-phenyl-2-thiohydantoin derivative of the hydroxy amino acid (XVll) was prepared by the technique described by Edman[23], crystallised from water as pale yellow prisms m.p. 171-172°. (Found : N, 10.8. $C_{13}H_{12}N_2O_2S$ requires N, 10.8%).

1-Amino-1-carboxypent-3(cis)-ene-5-ol (XVlll).- 1-Amino-1-carboxypent-3-yn-5-ol (1.086g.) dissolved in water (20cc.) was catalytically semi-hydrogenated over palladium-charcoal (10%, 0.05g.). The uptake of the theoretical volume of hydrogen (187cc., 18°/761mm.) being attained in two hours. The resulting hydroxy amino acid (0.8g., 73%) crystallised from methanol as a microcrystalline powder, m.p. 227° (decomp.). (Found : C, 49.6; H, 7.9; N, 9.7. $C_6H_{11}O_3N$ requires C, 49.4; H 7.6; N, 9.7%).

2-Amino-6-hydroxycaproic acid.- 1-Amino-1-carboxypent-3-ene-5-ol (200mg.) dissolved in water (10cc) was fully hydrogenated over Adams' catalyst (10mg.). The saturated hydroxy amino acid (120mg.) crystallised from water as needles, m.p. 254-260° (decomp.). Gaudry[24] gives m.p. 260-262° or 245-248° (decomp.) depending on the rate of heating. The phenylureido derivative crystallised from water as plates, m.p. 138°. Gaudry[24] gives

m.p. 141°. (Found : N, 10.8. Calc. for $C_{13}H_{18}O_4N_2$ N, 10.9%)

Summary of Ring Closure Attempts on 1-Amino-1-carboxy~~but~~pent-3(cis)-ene-5-ol (XVIII).

(a) Heating under reflux with concentrated hydrochloric acid for four hours and subsequent treatment with the anion exchange resin "Amberlite IR-4B" gave the starting material unchanged. Pleninger(19) successfully accomplished the ring closure of a closely similar saturated hydroxy acid to proline by this method. (See p. 37).

(b) Heating the methyl ester of the hydroxy amino acid for one hour, and, in a repeat experiment for six hours, at 100° followed by hydrolysis gave unchanged starting material.

(c) Heating at 200° with aniline furnished no anilide or ring closed product; only the hydroxy amino acid was isolated.

(d) The potassium salt of the hydroxy amino acid was dissolved in dimethylformamide which was azeotropically distilled to remove any water formed. After 3 hours only unchanged starting material was recovered.

(e) Treatment of the amino acid hydrochloride with phosphorous tribromide, cf. Bowden and Green(25), gave an intractable tar.

(f) Heating to the decomposition point in a high vacuum gave none of the sublimate expected if baikiain had been formed.

(g) Heating with sodium hydroxide (0.1N) and sodium carbonate (0.1N) solutions caused decomposition.

(h) Fusion with one equivalent of urea and heating the resulting melt at 130° for two hours followed by hydrolysis gave only unchanged starting material.

(i) Treatment with phosphorous pentoxide in triethylamine solution gave no isolatable product (See McElvain and Clarke[28]).

(j) A zinc chloride melt of the amino acid heated at 200° for four hours, followed by removal of the zinc chloride with ether, gave a high melting solid, insoluble in ether, water and ethanol but immediately soluble in dilute acids and alkalis. This product gave a positive test for zinc and chloride ions and was probably an amino acid-zinc chloride complex. On decomposition with acid and isolation of the amino acid, unchanged starting material (60%) was recovered.

(k) Heat treatment with ammonium carbonate gave the waxy amide of the amino acid (See Kao[29]). Hydrolysis of this amide gave the unchanged hydroxy amino acid.

NINHYDRIN REACTIONS OF THE AMINO ACIDS

Amino Acid	Decomp. Point.	5% Aqueous Ninhydrin	Citrate-buffered Ninhydrin Solution (a)
$NH_2-CH_2-C\equiv C-CH_2-CH(NH_2)COOH$	192-4°	Yellow	Violet
$NH_2-CH_2-\underset{H}{C}=\underset{H}{C}-CH_2-CH(NH_2)COOH.HCl$	257-8°	Yellow-brown	"
do. 2HCl	Hygros.	Yellow-brown	"
DO. H_2SO_4	237-8°	Yellow	"
$NH_2-(CH_2)_4-CH(NH_2)COOH.\ H_2SO_4$	-	Violet	"
$HO-CH_2-C\equiv C-CH_2-CH(NH_2)COOH$	192°	Brown	"
$HO-CH_2-CH=CH-CH_2-CH(NH_2)COOH$	227°	Yellow-brown (b)	"
$HO-(CH_2)_4-CH(NH_2)COOH$	254-9°	Violet	"

(a) Prepared as described by Moore and Stein (27)

(b) No violet colour even in the presence of 7% by weight of glycine.

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(21) Fraser & Raphael, J., 1952, 226.

(22) Von Braun, Ber., 1909, 42, 845.

(23) Edman, Acta Chem. Scand., 1940, 4, 277.

(24) Gaudry, Canad. J. Res., 1948, 26B, 387.

(25) Bowden & Green, J., 1952, 1164.

(26) Osterberg, Org. Syn., Coll. Vol. 1, 1932, 271.

(27) Moore & Stein, J. Biol. Chem., 1948, 176, 367.

(28) McElvain & Clarke, J.A.C.S., 1947, 69, 2657.

(29) Kao, J., 1931, 443.

PART 4

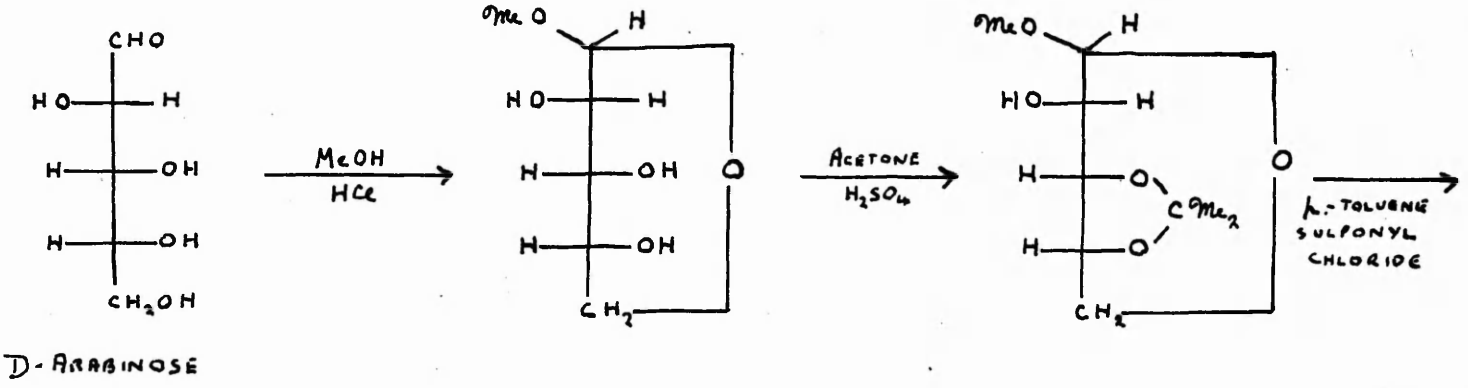
THE SYNTHESIS OF 2-DEOXY-D,L-RIBOSE

THE SYNTHESIS OF 2-DEOXY-D,L-RIBOSE

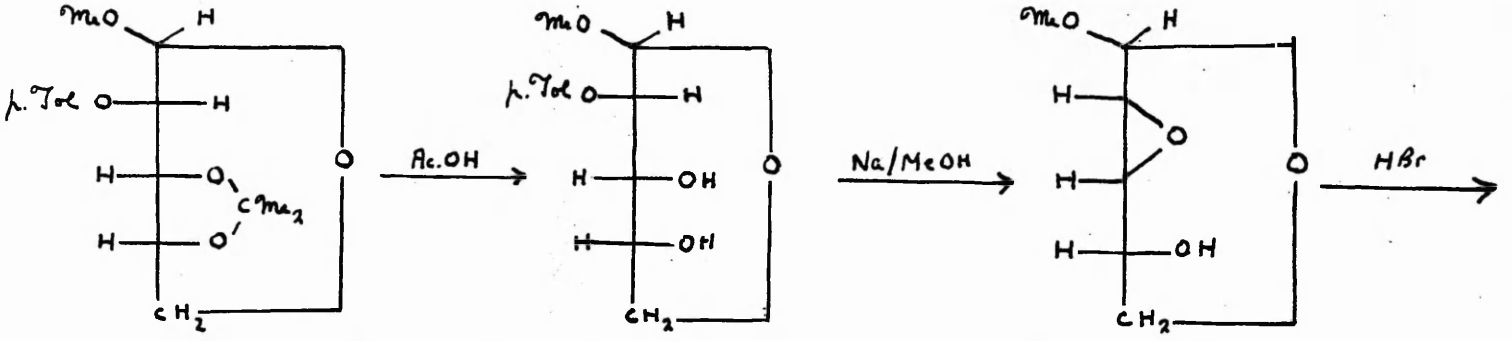
INDEX

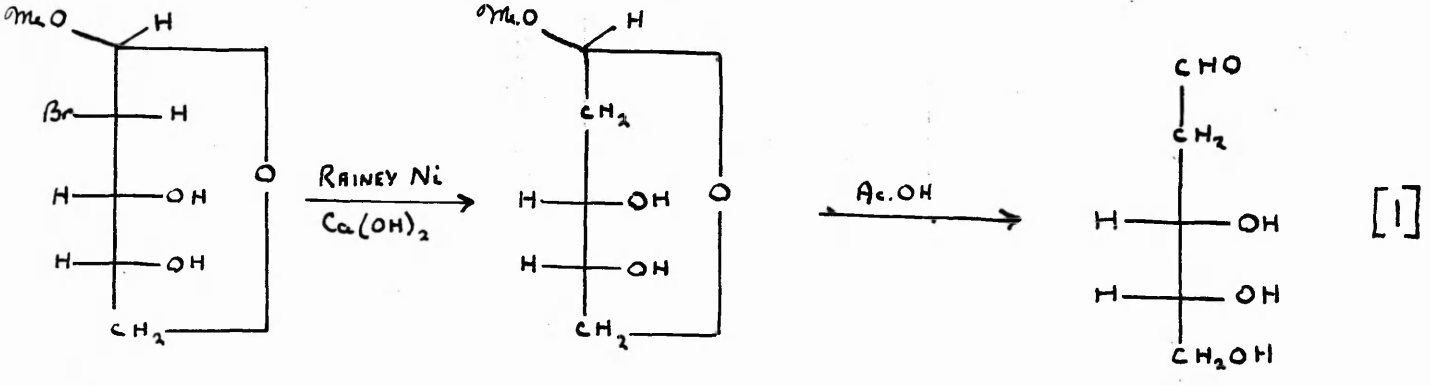
FLOW SHEET G

ROUTE BY KENT, STACEY AND WIGGINS



D-ARABINOSE



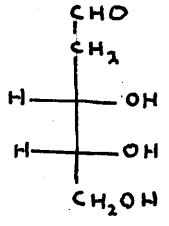


+ ISOMER

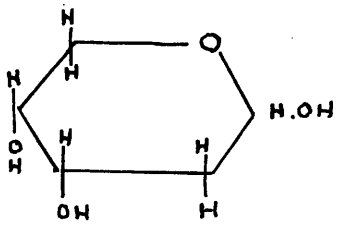
[I]

HISTORICAL

The history of the sugar 2-deoxy-D-ribose (1) can be



(1)



traced back to the second half of the 19th. Century, when the chemist first turned his attention to the constituents of the living cell. In 1869 the Swiss biochemist Miescher isolated a substance he called "nuclein" by the acid hydrolysis of pus cells[1]. Within a few months nuclein was isolated by Miescher and by other workers in the same laboratory from such varied natural sources as egg yolk[2], yeast cells[2], red blood cells of birds and reptiles[4] and casein[5]. The general physical properties of nuclein from these sources were identical but the elementary composition varied somewhat with the source.

Miescher now turned his attention to a more readily accessible source of nuclein; the spermatozoa of salmon. With workable quantities at his disposal he found that nuclein had two components, a polybasic organic acid and a basic substance. In 1889 Altmann[6] introduced the name "nucleic acid" for the acid portion of nuclein and confirmed the fact that nuclein consisted of nucleic acid and a protein. He described a general method of isolating nucleic acids free from protein.

Further work showed that the nucleus of every living

cell invariably consists of nuclein[7] (later named nucleo-protein). Nuclein has been isolated from the nuclei of a wide variety of biological matter, e.g., chromosomes, genes, viruses, bacteria and antigens[8].

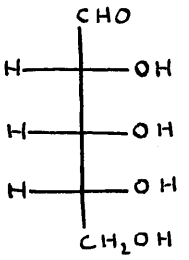
In 1891 Kossel[9] hydrolysed nucleic acid into its components and made the important discovery that there were two types of nucleic acid, differing only in certain components. Later investigators identified the hydrolysis products and by 1903 the following Table was drawn up.

Components of Nucleic Acids.

Type A. (e.g. from thymus gland)	Type B. (e.g. from yeast)	
Phosphoric Acid	Phosphoric Acid	
Adenine	Adenine	Purine and Pyrimidine Bases
Guanine	Guanine	
Cytosine	Cytosine	
5-Methyluracil	Uracil	
Sugar A	Sugar B	

The two types of nucleic acid can be seen to differ only in the sugar and the pyrimidine base uracil; the sugar being mainly responsible for the difference in physical properties. Other types of nucleic acid have been thought to occur but none have been identified in a convincing manner.

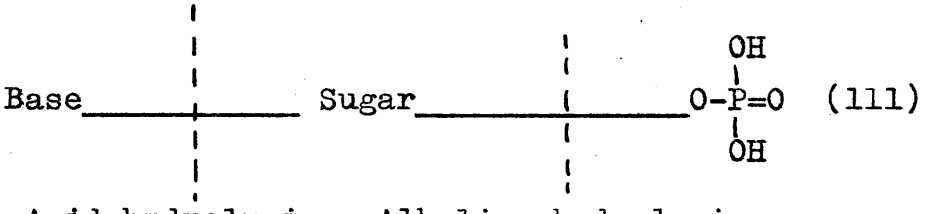
In 1909 Levene[10] identified sugar B as D-ribose (11).



(11)

Investigations, however, on the structure of sugar A were complicated by the instability of the sugar and the extreme solubilities of its derivatives (e.g. hydrazones, phenylhydrazones, etc.). The conditions used to hydrolyse nucleic acids to their separate components decomposed sugar A.

It was later discovered[11] that, by careful hydrolysis, nucleic acids could be broken down into four components, the "nucleotides" (111), each of which contained one molecule of

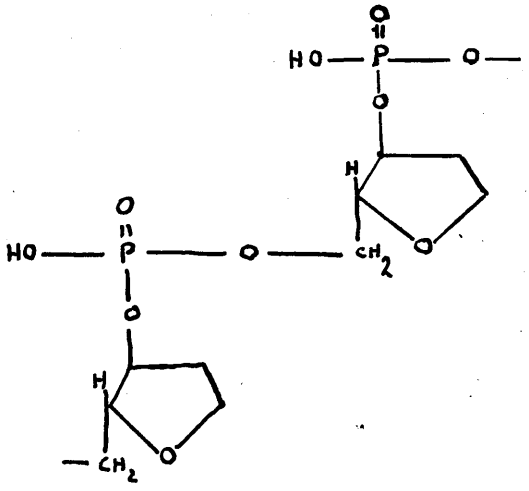


(111)

sugar, one molecule of phosphoric acid and one molecule of a purine or pyrimidine base. In 1929 Levene and London[12] succeeded in isolating guanine nucleotide (111, Base=Guanine, Sugar=A) from thymus gland tissue. Treatment of this nucleotide with dilute sulphuric acid (0.025N) gave the sugar phosphate portion free from the base. After removal of the phosphoric acid sugar "A" was at last obtained in a crystalline state. Analytical figures indicated the formula $C_5H_{10}O_4$. This result

and the fact that the sugar gave a 1:1-benzylphenylhydrazone but no ozazone indicated a 2-deoxyribose. The Dische test was carried out and the blue colour with diphenylamine, which is specific for 2-deoxypentoses[13] was obtained.

Levene[14] [15] now synthesised two 2-deoxypentoses, 2-deoxy-D-xylose and 2-deoxy-L-ribose, for comparison with sugar "A". It was found that sugar "A" and 2-deoxy-L-ribose had the same numerical values of initial and equilibrium specific rotation but had the opposite sign. Further comparisons confirmed the obvious deduction that sugar "A" was, in fact, 2-deoxy-D-ribose (1). Further work by Levene[16] showed that the sugar was present in the furanose form in deoxyribonucleic acid and the general structure of the latter was as depicted in (1V).



(1V)

Previous Syntheses of 2-Deoxyribose.

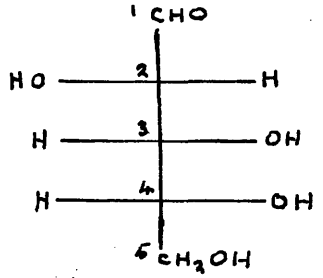
Previous synthesis of 2-deoxyribose can be classed under three headings[37].

(a) The Glycal Route.

(b) The Nitroolefin Route.

(c) Miscellaneous.

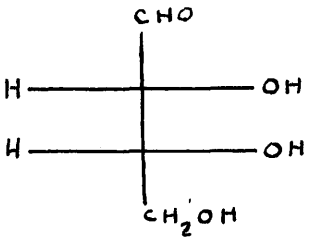
(a) The Glycal route (See Flow Sheet F, Opposite p. 55) has been thoroughly explored and many variations of this synthetic route have been tried out. The synthesis employs D or L-arabinose (V) as a starting material and consists essentially of replacing the 2-hydroxy of arabinose with hydrogen. The method was first used in 1927 by Meisenheimer and Jung(17) to prepare 2-deoxy-L-ribose and was



(V)

adapted by Levene and Mori(14) in 1929 to prepare this sugar for comparison with sugar "A" from thymus gland tissue. The overall yields, however, were very low (circa 1%). In 1935 Felton and Freudenberg(18) modified the techniques employed and increased the overall yield to 5%. More recently Deriaz et al(19) improved the process further (10% yield). Further work by Ohto in 1951(20) did not increase this figure.

(b) The Nitroolefin route (See Flow Sheet F, opposite p. 55) initially employs the C_4 sugar, D or L-erythrose (VI) and



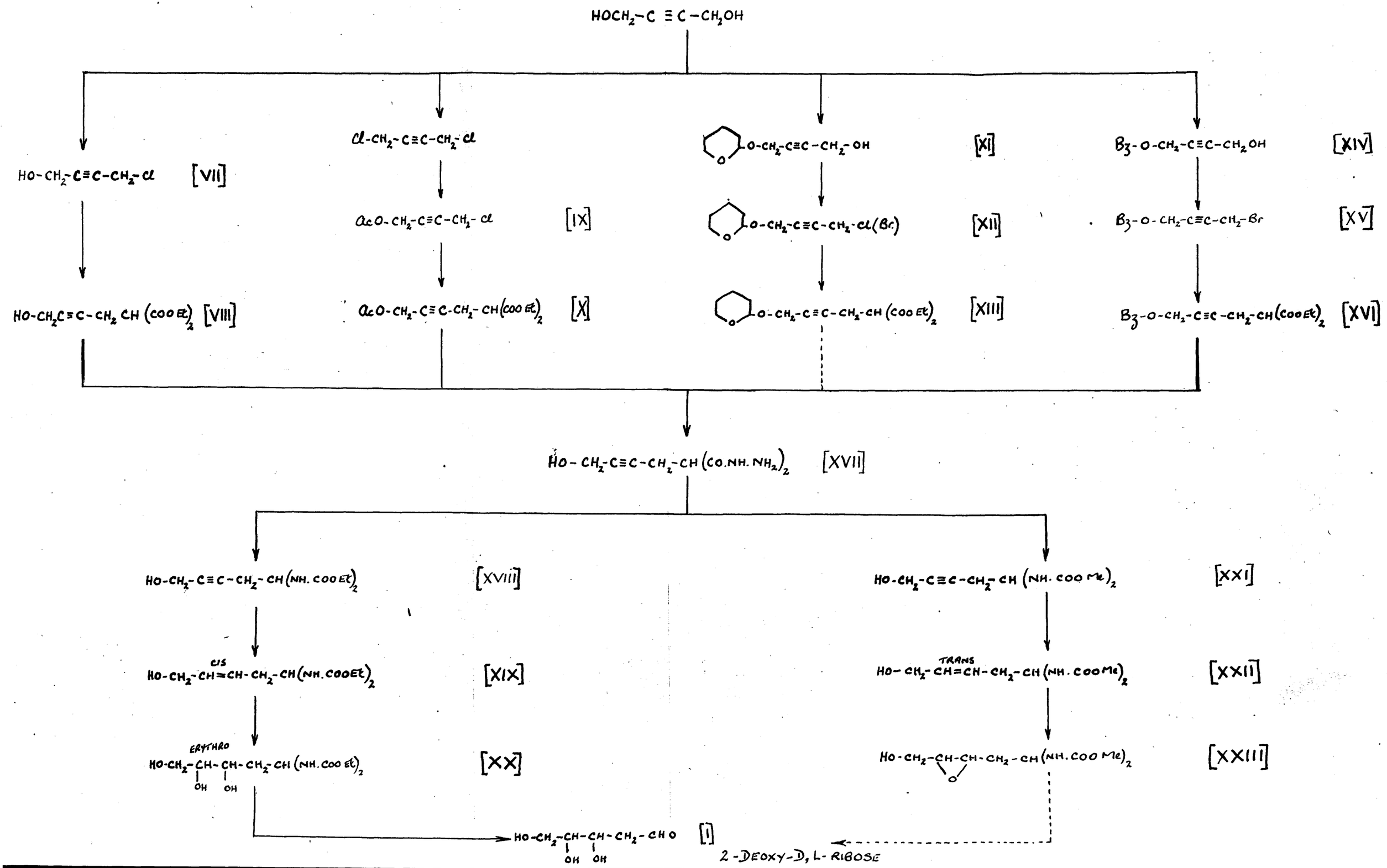
(VI)

builds up the requisite carbon skeleton by the addition of nitromethane to the aldehydic group, followed by dehydration, selective reduction of the double bond and Nef hydrolysis of the aci-nitroalcohol sodium salt. This method was employed by Overend, Stacey and Wiggins(21) in 1949 to prepare 2-deoxy-D-ribose. The overall yield was 0.5%. The method was improved by Sowden(22) who showed that overall yields of 20% could be obtained.

(c) Miscellaneous routes. In 1951 Hough(23) devised an ingenious synthesis of 2-deoxy-D-ribose from the C_3 sugar D-glyceraldehyde (See Flow Sheet F, opposite p.55). The overall yield was reported to be very small and no detailed instructions have, so far, been published.

Kent, Stacey and Wiggins(24) worked out yet another route to 2-deoxy-D-ribose from D-arabinose (Flow Sheet G, opposite p. 55.). The method can be seen to resemble the glycal route. The overall yield was circa 1%.

FLOW SHEET H


HOCH2-C ≡ C-CH2OH
HO-CH2-C≡C-CH2-Cl [VII]
HO-CH2C≡C-CH2 CH (COOEt)2 [VIII]
Cl-CH2-C≡C-CH2-Cl
AcO-CH2-C≡C-CH2-Cl [IX]
AcO-CH2-C≡C-CH2-CH(COOEt)2 [X]
O-CH2-C≡C-CH2-OH [XI]
O-CH2-C≡C-CH2-Cl(Br) [XII]
O-CH2-C≡C-CH2-CH (COOEt)2 [XIII]
BZ-O-CH2-C≡C-CH2OH [XIV]
BZ-O-CH2-C≡C-CH2-Br [XV]
BZ-O-CH2-C≡C-CH2-CH(COOEt)2 [XVI]
HO-CH2-C≡C-CH2-CH (CO.NH.NH2)2 [XVII]
HO-CH2-C≡C-CH2-CH (NH.COOEt)2 [XVIII]
HO-CH2-CH=CH-CH2-CH(NH.COOEt)2 [XIX]
HO-CH2-CH-CH-CH2-CH (NH.COOEt)2 [XX]
OH OH
HO-CH2-C≡C-CH2-CH (NH.COOMe)2 [XXI]
HO-CH2-CH=CH-CH2-CH (NH.COOMe)2 [XXII]
HO-CH2-CH-CH-CH2-CH (NH.COOMe)2 [XXIII]
HO-CH2-CH-CH-CH2-CHO [I]
OH OH
2-DEOXY-D, L-RIBOSE

DISCUSSION.

From the historical section it can be seen that previous syntheses of 2-deoxyribose all possessed two great disadvantages, viz., the overall yields were exceedingly small and the starting materials were comparatively inaccessible sugars. As has been recently noted(37) " a satisfactory synthesis of this important compound has clearly not yet been achieved ".

These facts indicated the necessity for further investigation on the synthesis of this important sugar and a new approach was envisaged using a non-carbohydrate starting material, the commercially available but-2-yn-1:4-diol, in a series of reactions delineated in Flow Sheet H, opposite p.61.

The first problem involved the conversion of but-2-yn-1:4-diol into 4-halogenobut-2-yn-1-ol or an ester derivative thereof. Theoretically the already achieved conversion of the glycol into 4-chlorobut-2-yn-1-ol (VII) (page 6) by the action of one mole of thionyl chloride in pyridine appears to be an attractive and direct route to such a derivative. Unfortunately the reaction is not only extremely temperamental as to yield but also the crude chlorobutynol tends to explode on being distilled and even when obtained pure was found to be a particularly insidious and efficient vesicant. For these reasons a more amenable derivative was sought.

Treatment of the readily preparable 1:4-dichlorobut-2-yne[26] with one mole of potassium acetate in glacial acetic acid furnished the expected mixture of dichlorobutyne, butynediol acetate and 4-chlorobut-2-yn-1-ol acetate (IX), the last named being easily isolated by fractional distillation. The comparatively low yield of the mono-acetate (25%), however, prompted the investigation of other derivatives. It had been already noted that the elegant reagent for the protection of the hydroxyl group, 2:3-dihydropyran, could be used to protect one hydroxyl group only of a symmetrical glycol[25]. Application of this reaction to butynediol resulted in an excellent yield of the required 4-(tetrahydropyronyloxy)but-2-yn-1-ol (XI); unfortunately replacement of the free hydroxyl group with thionyl chloride/ pyridine and phosphorous tribromide / pyridine resulted in poor yields of the expected 1-chloro and 1-bromo-4-(tetrahydropyronyloxy)but-2-ynes (XII).

Attempts at monoesterification of butynediol were then made. All efforts at partial acetylation failed but monobenzoylation, using a technique developed for the preparation of quinitol monobenzoate[31] proved much more successful. Treatment of the diol in chloroform with 85% of the theoretical one mole of benzoyl chloride produced the monobenzoate (XIV) in acceptable yield (61%). The action of phosphorous tribromide on the monobenzoate gave the required 1-benzoyloxy--4-bromobut-2-yne (XV) in an overall yield of 40% from butynediol. This undoubtedly constitutes the most workable

and reproducible process for obtaining the required type of compound from butynediol.

The condensation of these 4-halogenobut-2-yn-1-ol derivatives with diethyl malonate was then carried out. 4-Chlorobutynol (Vll) condensed readily with ethyl sodio-malonate in ethanol to produce the expected 1:1-dicarbethoxy-pent-3-yn-5-ol (Vlll) ; the acetate (X), the benzoate (XVl), and the tetahydropyronyloxy derivative (Xlll) of this compound were obtained by reacting the appropriate derivative with a suspension of ethyl sodio-malonate in toluene. Treatment of the alcohol (Vlll), the acetate (X) and the benzoate (XVl) with hydrazine produced the same dihydrazide (XVll) from all these compounds ; the acetohydrazide and benzhydrazide formed as by-products from the last two starting materials were easily separable from the dihydrazide (XVll) as this product was found to be very sparingly soluble in ethanol.

Treatment of the dihydrazide with nitrous acid followed by the reaction of the resulting diazide with ethanol[27] gave the corresponding diurethane, 1:1-(dicarbethoxyamino) pent-3-yn-5-ol (XVlll). As this compound proved rather difficult to crystallise the corresponding methyl urethane 1:1-(dicarbmethoxyamino)pent-3-yn-5-ol (XXl) was prepared by treating the diazide with methanol ; this product was indeed more readily crystallisable but, rather unexpectedly possessed a lower melting point than the ethyl analogue.

Partial catalytic hydrogenation over palladium, which

is known to confer the cis configuration(28), of the ethyl urethane (XVIII) gave the crystalline 1:1-(dicarbethoxyamino) pent-3-ene-5-ol (XIX). A similar reduction carried out on the methyl ana-logue (XXII) gave an uncrystallisable oil. Osmium tetroxide catalysed cis-hydroxylation of this product with hydrogen peroxide(29) gave the erythro-triol, 1:1-(dicarbethoxy-amino) penta-ne-3:4:5-triol (XX) as a viscous glass ; the erythro configuration of this product follows from many previous precedents(39). The glassy triol was then treated with dilute barium hydroxide at room temperature. Removal of the barium and consequent evaporation gave a glassy product which was continually extracted to remove any ether-soluble by-pro-ducts. The residue consisted of a hard glass which gave a positive Fehlings reaction and an exceedingly strong Dische test. This latter test, the production of a blue colouration by heating with diphenylamine in acetic acid, has been found to be specific for 2-deoxypentoses(13). There seems little doubt that this product consisted essentially of 2-deoxy-D,L-ribose (1) but all attempts to crystallise the glassy material failed ; attempts to prepare the anilide and benzylphenyl-hydrazone yielded only uncrystallisable oils. Use of dilute sodium hydroxide to effect the final hydrolysis led to immed-iate tar formation.

Although 2-deoxyribose is sensitive to acids, the end product of interaction being laevulinic acid, its derivatives have been successfully hydrolysed to the parent sugar by mild

acid treatment[22][24]. Accordingly attempts were made to hydrolyse the triol diurethane (XX) to 2-deoxyribose with dilute sulphuric acid at room temperature; this procedure yielded only unchanged starting material. Use of higher temperature yielded a product, giving a green Dische test, the nature of which was not further investigated. Deriaz et al[13] and Pirie[30] have shown that certain aldehydes, e.g. aldol, glyceraldehyde, furfuraldehyde and hydroxymethyl-furfuraldehyde give a green colour with Dische reagent. This indicated that although the warm acid was causing hydrolysis of the diurethane grouping it was also modifying the remainder of the molecule. Use of a hot aqueous suspension of an acidic ion exchange resin (Amberlite IR-120) in an attempt to effect the hydrolysis yielded only unchanged starting material.

An alternative approach to 2-deoxyribose was then attempted, starting from the acetylenic methyl diurethane (XXI). The method envisaged the chemical partial reduction of (XXI) to the corresponding trans-ethylenic derivative followed by trans-hydroxylation to the erythro-triol diurethane. The most elegant method for reducing triple bonded compounds to the corresponding trans-ethylenes involves their interaction with sodium in liquid ammonia[28][32]. However when this process was applied to the acetylenic diurethane (XXI) the reaction used up far more sodium than

was theoretically neccessary and the product was found to be a mixture of the required trans-1:1-(dicarbomethoxyamino) pent-3-en-5-ol (XXll) and a less oxygenated compound which proved to be (XXlV). The removal of an allylic hydroxyl group by sodium in liquid ammonia has been noted before ;

$$\overset{\text{trans}}{CH_3\text{-}CH{=}CH\text{-}CH_2\text{-}CH(NH.COOMe)_2} \qquad \text{(XXlV)}$$

it has also been reported that this hydrogenolysis may be prevented by preforming the sodium salt of the alcohol(38). Accordingly one mole of sodamide was added to the liquid ammonia solution of the acetylenic diurethane (XXl) before the introduction of the metallic sodium. This technique yielded only the required trans-ethylenic diurethane (XXll), uncontaminated with the hydrogenolysis product.

Trans-hydroxylation of (XXll) was first attempted using the Prévost reagent(33) a process involving essentially of the trans addition of two benzoyloxy groups to the double bond. Addition was very slow and furnished a mixture of unchanged starting material and an uncrystallisable glassy product.

It was then found that the trans-ethylenic diurethane (XXll) reacted smoothly with one mole of perbenzoic acid in chloroform to give the corresponding crystalline epoxide (XXlll). It was hoped that treatment of this product with

dilute sulphuric acid would both hydrolyse the urethane groupings and convert the epoxide ring into a diol, thus furnishing 2-deoxyribose directly. Unfortunately the product from this procedure, a yellow glass, gave a dull red colour turning rapidly to green with the Dische reagent and it is thus unlikely to have contained an appreciable amount of 2-deoxyribose. An attempt to convert the epoxide (XXlll) to the corresponding triol by heating with water alone(34) unexpectedly caused deep-seated decomposition, the product again giving an atypical Dische Test.

Summarising the above work, the best route found to 2-deoxy-D,L-ribose from butynediol was <u>via</u> the monobenzoate (XlV) to the corresponding bromobenzoate (XV) : condensation of which with malonic ester and subsequent treatment with hydrazine yielding the key intermediate, 1:1-di(hydrazidocarbonyl)pent-3-yn-5-ol (XVll). From what has been found above, the <u>trans</u> hydrogenation-<u>trans</u> hydroxylation route to the <u>erythro</u> configuration does not seem at all promising. The other route involving <u>cis</u> hydrogenation-<u>cis</u> hydroxylation to erythro 1:1-(dicarbethoxyamino)pentane-3:4:5-triol (XX), followed by treatment with dilute barium hydroxide appears to furnish the required 2-deoxy-D,L-ribose.

EXPERIMENTAL

1-Chlorobut-2-yn-4-ol (V11).- This compeund and its phenylurethane derivative were prepared earlier (see p. 6). The halide being recovered as a by-product from the preparation of 1:4-dichlorobut-2-yne.

But-2-yn-1:4-diol (70g.; 1 mol.) was dissolved in dry pyridine (100cc.) and the solution cooled to 5°. Thionyl chloride (64.5cc.; 1.1 mol.) was added dropwise over one hour with stirring, the temperature being kept between 5° and 10°. The mixture was then allowed to reach room temperature and stirred for a further 6 hours. Crushed ice was added and the product isolated with ether (5 x 100cc.). The combined ether extracts were washed with dilute sulphuric acid (1N), dilute sodium carbonate and water, keeping the aqueous volumes as low as possible, and dried over sodium sulphate. After removal of the ether the crude product was fractionated at 12 mm. 1:4-dichlorobut-2-yne (15g.; 15%) distilled over first at 63-65°, followed by 1-chlorobut-2-yn-4-ol (V11) at 87-88°. When 24g. (27%) of this monohalide had distilled the distillation flask exploded. White fumes were noted coming from the heated mixture (oil bath temp.= 140°) 6-8 seconds before the explosion. 1-chlorobut-2-yn-4-ol was a clear liquid, n_D^{19} 1.5002, rapidly turning yellow at room temperature

and ultimately polymerising to a black tar in a few weeks ; this decomposition was much slower if the product was kept at 0°. As reported earlier it was found to have vesicant properties. This compound exploded again on distillation at 12 mm. during a repeat of the preparation (oil bath temp. = 120°). In another preparation the monohalide was distilled safely at 0.1 mm., b.p. 47-48°.

1:4-Dichlorobut-2-yne.- This compound was prepared by the action of 2.4 moles of thionyl chloride in pyridine as described by Johnson(26).

1-Chlorobut-2-yn-4-ol acetate (1X).- 1:4-Dichlorobut-2-yne (16.5g.; 1 mol.) and potassium acetate (13.1g.; 1 mol.) were dissolved in glacial acetic acid (50 cc.) and the solution heated under reflux for 21 hours. The precipitated potassium chloride (5.34g.; theor. 9.9g.) was filtered off and the bulk of the acetic acid removed by distillation.The residue was poured into water (50 cc.) and the product extracted with ether; the combined ether extracts were washed with dilute sodium bicarbonate and water, then dried over sodium sulphate. After removal of the solvent the product was fractionated to give the required acetate of 1-chlorobut-2-yn-4-ol (1X) (4.6g.; 23%) as a clear oil, b.p. 98-100°/14 mm., n_D^{20} 1.4720. (Found : C, 49.24; H, 4.9. $C_6H_7O_2Cl$ requires C, 49.17; H, 4.8%) .

Other fractions were the starting material 1:4-dichloro-but-2-yne (1.5g.), b.p. 64-66°/14 mm.; and the diacetate of but-2-yn-1:4-diol (2g.), b.p.129-130°/14 mm., n_D^{20} 1.4596. Johnson[26] gives b.p. 122-123/10 mm., n_D^{20} 1.4611 .

A similar condensation was carried out in methyl ethyl ketone, heating under reflux for 12 hours. Starting material (90%) was recovered with a small amount of the required monoacetate. Even with longer heating times yields higher than 25% could not be obtained. On heating under reflux in acetic acid for 36 hours the yield of diacetate increased, the yield of monoacetate remaining almost constant (20%). The use of anhydrous sodium acetate in place of potassium acetate gave identical results.

4-(Tetrahydropyranyloxy)but-2-yn-1-ol (XI).- c.f.[25]. But-2 -yn-1:4-diol (34.6g.) and six drops of concentrated hydrochloric acid were heated gently until the diol was molten. 2:3-Dihydropyran (34g.) was then added dropwise to the stirred solution over one hour, the heat developed by the reaction keeping the mixture molten. After standing overnight the product was dissolved in ether, washed with dilute sodium bicarbonate and water and the ether extract dried over sodium sulphate. After removal of the solvent fractionation of the crude product gave the monoaddition product as a low melting solid (56.53g.; 82%), m.p. 20-25°, b.p. 142-144°/14 mm., 109-111°/0.05 mm., n_D^{24} 1.4873.

(Found : C, 64.0; H, 7.6. $C_9H_{14}O_3$ requires C,63.5; H,8.3%).

1-Chloro-4-(tetrahydropyranyloxy)but-2-yne (XII) .- The above pyranyl ether (6.82g.) was dissolved in dry pyridine (15 cc.) and the mixture cooled to 0°. Thionyl chloride (4.4 cc.) was added dropwise with stirring, the temperature being kept between 5-10°. After standing 2½ hours at room temperature the mixture was poured into water containing crushed ice. The product was extracted with ether, the ethereal extract washed with dilute sulphuric acid, dilute sodium bicarbonate and water. The ether extract was then dried over sodium sulphate. After removal of the ether the crude product was fractionated to give 1-chloro-4-(tetrahydropyranyloxy)but-2-yne (2.05g.; 29%) as a clear oil b.p. 92-94°/13 mm., n_D^{20} 1.4912. (Found C, 57.0; H, 7.0. $C_9H_{13}O\,Cl$ requires C, 57.3; H, 7.0%). 1;4-Dichlorobut-2-yne (1g.) was recovered from the first fractions. After fractionation there was a large tarry residue (3g.).

1-Bromo-4-(tetrahydropyranyloxy)but-2-yne (XII) .- See Eglington and Whiting(35). The above pyranyl ether, 4-(tetrahydropyranyloxy)but-2-yn-1-ol (8g.) was dissolved in a mixture of dry ether (50 cc.) and dry pyridine (10 cc.). Phosphorous tribromide (2 cc.) in dry ether (15 cc.) was added slowly to the stirred mixture. The solution was left at room temperature overnight. The ether layer was then

washed with dilute sulphuric acid, dilute sodium bicarbonate and water and finally dried over sodium sulphate. After the removal of the ether fractionation of the crude residue gave 1-bromo-4-(tetrahydropyranyloxy)but-2-yne (3.1g.; 30%) as a clear oil, rapidly turning at room temperature to yellow, b.p. 70°/0.05 mm., n_D^{24} 1.5246. (Found C, 46.2; H, 5.5. $C_9H_{13}O$ Br requires C, 46.4; H, 5.6%). 1:4-Dibromobut-2-yne (1.2g.) was recovered from the first fractions and again there was a large tarry residue (4 cc.).

But-2-yn-1:4-diol monoacetate .- Attempts to prepare this compound directly from but-2-yn-1:4-diol were unsuccessful The following results were obtained :-

(a) The action of one mole of acetyl chloride in a chloroform/pyridine solution of the diol gave a 60% yield of the diacetate. No monoacetate was recovered, the small quantity present being probably removed when washing the chloroform solution with water.

(b) The diol was heated under reflux for five hours with one mole of acetic acid in an ethyl acetate-chloroform mixture plus a few drops of concentrated sulphuric acid. 80% of the diol was recovered unchanged.

(c) The diol heated with one mole of acetic anhydride gave a 90% yield of the diacetate. Similar treatment in ethyl acetate solution gave a 76% yield of the diacetate.

(d) Attempts to partially hydrolyse the diacetate with

hydrochloric and hydrobromic acids gave a mixture from which only the diol and its diacetate could be isolated.

1-Benzoyloxybut-2-yn-4-ol (XlV) .- The method employed is a modification of that used by Jones and Sondheimer(31) for the preparation of quinitol monobenzoate.

But-2-yn-1:4-diol (95g.) was dissolved in a mixture of dry chloroform (250 cc.) and dry pyridine (110 cc.). The mixture was cooled to 0° and benzoyl chloride (110 cc.), dissolved in dry chloroform (200 cc.), was added, with stirring, over four hours, the temperature being kept below 5°. The solution was then allowed to reach room temperature and stirring was continued for four hours. The chloroform solution was then extracted with sulphuric acid (1N ; 4 × 100 cc.) and water (3 × 100 cc.). After the chloroform solution had been dried over sodium sulphate, ethanol (150 cc.) was added and the mixture kept at -16° overnight. Crystalline but-2-yn-1:4-diol dibenzoate (45g.), m.p.75-76° was then filtered off. Johnson(26) gives m.p. 76-77° for the dibenzoate. After removal of the solvent from the filtrate the residue of crude monobenzoate was fractionated to give the pure monobenzoate of but-2-yn-1:4-diol as a colourless oil, (101.8g.; 61%), b.p. 117°/8 × 10^{-5}mm., n_D^{19} 1.5510. (Found : C, 69.46; H, 5.23. $C_{11}H_{10}O_3$ requires C, 69.21; H, 5.42%)

In this preparation only 85% of the theoretical amount of benzoyl chloride was used. Any increase on this figure

resulted in a lessening of the amount of monobenzoute and produced an increase in the yield of dibenzoate. A decrease of this amount lowered the yield of both mono and dibenzoates.

The phenylurethane derivative of the monobenzoate crystallised as clusters of fine needles, m.p. 120°, from benzene-petrol (b.p. 60-80°). (Found : N, 4.6. $C_{18}H_{15}O_4N$ requires N, 4.5%)

1-Benzoyloxy-4-bromobut-2-yne (XV).- 1-Benzoyloxybut-2-yn-4-ol (70g.) was dissolved in dry benzene (1000 cc.). Phosphorous tribromide (25 cc.) was then added with stirring, producing a slight rise in temperature ;, stirringwas continued, at room temperature, for 24 hours. Iced water was then added and the mixture extracted with ether (3 × 500 cc.). The combined ether extracts, after being washed with dilute sodium bicarbonate solution and water, were dried over sodium sulphate. After removal of the solvent the crude product was fractionated to give 1-benzoyloxy-4-bromobut-2-yne (75g.;81%) as a clear liquid turning yellow at room temperature, b.p. 104-106°/8×10^{-5} mm, n_D^{18} 1.5744. (Found : C, 52.36; H, 3.8. $C_{11}H_9O_2Br$ requires C, 52.2; H, 3.6%). This bromobenzoate had an irritant action on the skin.

1:1-Dicarbethoxypent-3-yn-5-ol acetate (X) .- Ethyl malonate (6.86g.) was added dropwise with stirring to atomised sodium (0.99g.) in toluene (50 cc.). The vigourously stirred sol-

ution was heated for four hours at 50°. The acetate of 1-chlorobut-2-yn-4-ol (6.28g.) was added dropwise over 20 minutes and the solution heated at 80° for eight hours. The toluene layer, when cool, was washed with dilute sulphuric acid (2N) and water ; after drying over sodium sulphate the solvent was distilled off and the product fractionated to give 1:1-dicarbethoxypent-3-yn-5-ol acetate (X) as a colourless oil (3.6g.; 30%), b.p. 98-99°/1 × 10^{-4} mm, n_D^{22} 1.4549. (Found : C, 57.63; H, 6.67. $C_{13}H_{18}O_6$ requires C, 57.77; H, 6.71%) . The low yields obtained in this condensation were attributed to sodium chloride forming a crust round the particles of ethyl sodio-malonate, thus preventing further action.

1:1-Dicarbethoxy-5-(tetrahydropyranyloxy)pent-3-yne (XIII).- The condensation of the pyranyl ether bromide (XII) with ethyl sodio-malonate in ethanol gave the condensation product in approx. 50% yield, b.p. 154-157°/3 × 10^{-4}mm., This product however could not be purified satisfactorily due to extensive foaming during distillation. This difficulty and the poor yields of halide obtained led to the abandonning of this route.

1:1-Dicarbethoxy-5-benzoyloxypent-3-yne (XVI) .- Ethyl malonate (16g.) was added dropwise, with stirring, to toluene (250 cc.) containing atomised sodium (2.3g.). The stirred solution was heated for four hours at 50o.

1-Benzoyloxy-4-bromobut-2-yne (25.3g.) was then added over 30 minutes, the temperature being kept at 50°. When addition was complete the solution was heated at 100° for 16 hours. The cooled solution was washed with dilute sulphuric acid (1N.), dilute sodium carbonate solution and water. After drying over sodium sulphate the toluene was removed in vacuo and the residue fractionated to give 1:1-dicarbethoxy-5-benzoyloxypent--3-yne (XVI) as a clear oil, (22.5g.; 77%), b.p. 142-144°/ 8×10^{-5}mm., n_D^{20} 1.5056. (Found : C, 64.75; H, 5.72. $C_{18}H_{20}O_6$ requires C, 65.05; H, 6.06%)

1:1-Di(hydrazidocarbonyl)pent-3-yn-5-ol (XVII) .-

A. From 1:1-dicarbethoxypent-3-yn-5-ol (VIII)

The crude dicarbethoxypentynol (48.2g.) used was prepared as follows:- Sodium (5.83g.;1.1 mol.) was dissolved in dry ethanol (250 cc.) and ethyl malonate (40.48g.; 1.1 mol.) added. The solution was heated for two hours at 50°; 1-chlorobut-2-yn-1-ol (24g.; 1 mol.) in dry ethanol (50 cc.) was added dropwise to the stirred mixture, the temperature being kept below 50°. The solution turned red and sodium chloride precipitated out. When addition was complete (50 min.) the stirred solution was heated under reflux for 13 hours after which the bulk of the ethanol was distilled off and the residue poured into water. The product was extracted with ether, the extract washed with dilute sulphuric acid (1N), dilute sodium carbonate solution then water, and dried

over sodium sulphate. After removal of the ether the low boiling fractions were removed by distillation at 0.2 mm.. When the temperature of the distillate had reached 120° the distillation was stopped, leaving the crude 1:1-dicarbethoxypent-3-yn-1-ol (VIII) as a red viscous liquid.

This crude product was mixed with an equal volume of 100% hydrazine hydrate and heated on an oil bath at 120° for 20 minutes, with vigourous shaking. Boiling ethanol (500 cc.) was added followed by hot water until all the precipitated hydrazide passed into solution. On standing at 0° 1:1-di-(hydrazidocarbonyl)pent-3-yn-5-ol (XVII) crystallised out as fine needles, (27.45g.; 46%), m.p. 141-143°. Recrystallisation from aqueous ethanol (90%) gave m.p. 145°. (Found : C, 42.27; H, 5.89. $C_7H_{12}O_3N_4$ requires C, 41.99; H, 6.05%). The hydrazide was very soluble in water, sparingly soluble in chloroform and ethanol and insoluble in ether, petrol and carbon tetrachloride.

A small quantity of the tetrahydrazide of 1:1:6:6-tetracarboxyhex-3-yne was obtained as a by-product. It was insoluble in ethanol and crystallised from aqueous ethanol (80%) as needles, m.p. 211° (decomp.). (Found : N, 35.2. $C_{10}H_{18}O_4N_8$ requires N, 35.6%). This compound was probably formed from traces of 1:4-dichlorobut-2-yne in the starting material 4-chlorobut-2-yn-1-ol.

B. From 1:1-dicarbethoxypent-3-yn-5-ol acetate (X).-

1:1-Dicarbethoxypent-3-yn-5-ol acetate (2.4g.) was mixed with an equal volume of 100% hydrazine hydrate. The mixture was heated, with shaking, at 120° until it became homogeneous (3 mins.) and then heated for a further 2 minutes at the same temperature. Additional heating was found to decrease the yield. Boiling ethanol (50 cc.) was added and the solution cooled to 0°. The precipitated hydrazide was filtered off and recrystallised from aqueous ethanol (90%) as fine needles (1.28g.; 72%), m.p. 145°.

C. From 1:1-dicarbethoxypent-3-yn-5-ol benzoate (XVI).- The above benzoate (XVI) (21.2g.) was heated for 8 minutes at 120° with an equal volume of 100% hydrazine hydrate. Boiling ethanol (300cc.) was added and the flask cooled to 0°. The precipitated hydrazide crystallised from aqueous ethanol (90%) as fine needles (8.68g.; 68%), m.p. 145°.

1:1-(Dicarbethoxyamino)pent-3-yn-5-ol (XVIII).- This diurethane was prepared by the method described by Curtius and Mott (27)

The above hydrazide (XVII ; 3.78g.) was dissolved in dilute sulphuric acid (conc. sulphuric acid 2.01cc.; water 16.8cc.) and a top layer of ether (40cc.) added. The mixture was cooled to -5° and a solution of sodium nitrite (3.94g.) in water (10cc.), cooled to -5°, was added dropwise, with stirring, over 15 minutes; the temperature being kept below 0°. Ice-cold water was added to dissolve the precipitated salts and after 10 minutes stirring the ether layer was

decanted off and the green solution extracted with further quantities of ether (3 × 50cc.). The combined ether extracts containing the diazide were quickly washed with a little water and dried at 0° over freshly fused sodium sulphate. Ethanol (50cc.) was then added to the filtered ether solution, the ether was distilled off and the resulting ethanolic solution heated under reflux for three hours. On removal of the ethanol the resulting crude diurethane rapidly solidified and crystallised from a chloroform-carbon tetrachloride mixture (1:4) as tiny prisms (1.8g.; 37%), m.p. 138°.
(Found : C, 51.01; H, 7.04; N, 10.78. $C_{11}H_{18}O_5N_2$ requires C, 51.15; H, 7.02; N, 10.85%)

In this preparation the theoretical amounts of sodium nitrite and sulphuric acid were used. In repeat experiments excess sodium nitrite was used with minor variations in time and temperature of reaction. The yields obtained, however, ranged from 35 to 40%.

1:1-(Dicarbmethoxyamino)pent-3-yn-5-ol (XXl).- This diurethane was prepared by modifying the method of Curtius and Mott(27).

The above hydrazide (XVll ; 10.7g.) was dissolved in dilute sulphuric acid (conc.sulphuric acid, 5.7cc.;water,50cc.) and a top layer of ether (60cc.) added. The mixture was cooled to -8°; excess sodium nitrite (15g.) dissolved in water (30cc.) was cooled to -8°, and added dropwise, over 15 minutes, to the hydrazide solution, the temperature not being allowed to rise

above -4° ; the flask was vigourously shaken during the addition. The mixture was allowed to stand five minutes at 0°, then the green ether layer was decanted off. As rapidly as possible the semi-solid mixture in the reaction flask was shaken with fresh portions of ether at 0°, (5 × 50cc.), the decanted ether layers being kept at 0°. The combined ether extracts were washed once quickly with ice-cold water (50cc.) and freshly fused sodium sulphate added. The ether extract was allowed to dry at 0° for 30 minutes, filtered and methanol (100cc.) added. The ether was removed by ~~filtration~~ distillation and the resulting methanolic solution was heated under reflux for four hours. On removal of the solvent the crude product (6.5g.) was recrystallised from ethyl acetate to give 1:1-(dicarbmethoxyamino)pent-3-yn-5-ol (XXI) as rosettes of fine needles (5.56g.; 84%), m.p. 116°. (Found : C, 47.21; H, 5.80; N, 12.37. $C_9 H_{14} O_5 N_2$ C, 46.95; H, 6.12; N, 12.17%).

cis 1:1-(Dicarbethoxyamino)pent-3-en-5-ol (XIX).- 1:1-(Dicarbethoxyamino)pent-3-yn-5-ol (7.37g.) was catalytically semi-hydrogenated in ethyl acetate (150cc.) over 10% palladium-charcoal (0.4g.). The theoretical volume of hydrogen (803cc., 21°/762mm.) was absorbed in 30 minutes. After removal of the catalyst and solvent the crude product was suspended in boiling petrol (b.p. 60-80°) and ethyl acetate added till solution was effected. The cis-ethylenic diurethane (5.1g.; 69%) crystallised as needles, m.p. 92°. (Found : C, 70.75;

H, 7.70. $C_{11}H_{20}O_5N_2$ requires C, 70.75; H, 7.74%).

erythro-1:1-(Dicarbethoxyamino)pentane-3:4:5-triol (XX).- The above cis-ethylenic diurethane (XIX ; 0.5g.) was dissolved in a solution of hydrogen peroxide in tert-butanol (0.8cc.; 2.5M ; 0.77cc. theoretical). After cooling the mixture in an ice bath two drops of a 0.5% solution of osmium tetroxide in tert-butanol were added. An orange colour developed almost immediately. The solution was kept at 0° for 20 hours and the tert-butanol then removed in vacuo leaving the triol as a hard glass. This glass resisted all attempts at crystallisation. It was very soluble in water, acetone and the lower alcohols ; moderately soluble in ethyl acetate and insoluble in benzene, ether, chloroform and petrol. This preparation was repeated five times with variations in time and amounts of hydrogen peroxide and osmium tetroxide. Similar glasses were obtained from these preparations. The glasses gave a stable red colour with Dische reagent.

An attempt to prepare the tribenzoate of the triol by the action of benzoyl chloride in dry pyridine at room temperature gave a glass from which benzoic anhydride, m.p. 39°, was the only crystalline product. An attempt to prepare the triacetate by the action of acetic anhydride gave another intractable glass.

Hydrolysis of erythro 1:1-(dicarbethoxyamino)pentane-3:4:5-triol

A. Basic Hydrolysis. The glass obtained by hydroxylation of the ethylenic diurethane (0.5g.) above was dissolved in barium hydroxide solution (150cc.; 0.0665N) and the resulting solution kept stoppered at 40° for 20 hours ; slow precipitation of barium carbonate was observed. Carbon dioxide was then passed into the solution and the precipitated barium carbonate filtered off. The water was then removed under reduced pressure ; a further quantity of barium carbonate precipitating out was removed by filtration. When the solution was reduced in volume to 0.5cc. the remaining water was removed over phosphorous pentoxide in a vacuum desiccator. A hard glass was left which reduced Fehlings' solution and gave an intense stable blue colour with Dische reagent. The glass was extracted with boiling dry ethyl acetate, the bulk of the glass dissolved leaving a small quantity of a hygroscopic solid, m.p. 200-250° (decomp.), which was soluble in water and insoluble in ethanol. This solid, which charred on ignition, gave a solid residue which gave a green barium flame test. A solution of this solid in water gave a precipitate of barium sulphate on addition of dilute sulphuric acid. The solid also gave a faint blue colour with Dische reagent.

On removal of the solvent from the ethyl acetate extract a clear hard glass was obtained which gave an intense deep blue colour with Dische reagent. Attempts to crystallise this

compound from iso-propanol and ethyl acetate failed. Repeated trituration with different solvents and storage over phosphorous pentoxide in a vacuum desiccator failed to provide a seed of D,L-2-deoxyribose (1).

Attempts to prepare the anilide of 2-deoxyribose as described by Kent et al(24) and the benzylphenylhydrazone as described by Sowden(22) gave only oils from which crystalline derivatives could not be obtained.

An attempt to hydrolise the diurethane triol with sodium hydroxide solution (0.1 N.) in the cold produced decomposition.

B. Acid Hydrolysis. Attempts to hydrolise the diurethane triol (XX) with sulphuric acid.

(1) 0.01 N and 0.1 N sulphuric acid at room temperature gave the original glass unchanged. After removal of the sulphuric acid the residual glass gave a red colour with Dische reagent.

(2) On heating at 50° for ten minutes with 0.01N and 0.1N sulphuric acid decomposition occurred, the product giving a dark green colour with Dische reagent.

From these results it seems that the conditions required to hydrolise the diurethane group with sulphuric acid were drastic enough to decompose the sugar.

(3) An attempt was made to hydrolise the sugar using an insoluble acid catalyst. The glassy diurethane triol was heated at 80° for three hours with Amberlite Resin IR-120, the product after removal of the resin and water was the original glass giving a red colour with Dische reagent.

trans 1:1-(Dicarbomethoxyamino)pent-3-en-5-ol (XXII).- The corresponding acetylenic diurethane (XXI ; 5.06g.; 1 mol.) was finely powdered and dissolved in liquid ammonia (600cc.) held in a well insulated flask (1000cc.) equipped with a stirrer. Finely ground sodamide (0.85g.; 1 mol.) was added in portions to the stirred solution. After ten minutes sodium (2.02g.; 2 mol.) was added in small pieces over fifteen minutes. The stirred solution was allowed to stand eight hours, excess sodium (total 1g.) being added from time to time when the blue colour of the solution disappeared. Ammonium chloride (10g.) was added to the liquid ammonia solution and the ammonia allowed to boil off overnight. The solid residue in the flask was extracted with boiling ethyl acetate (3 × 20cc.). On removal of the ethyl acetate the residual crude trans-ethylenic diurethane (XXII) was crystallised from a light petroleum (b.p. 60-80°)-ethyl acetate mixture (1:1) from which it formed large prisms (3.03g.; 60%), m.p. 101-102°. (Found : C, 46.46; H, 6.71. $C_9H_{16}O_5N_2$ requires

C, 46.54; H, 6.95%). Quantitative microhydrogenation showed one double bond to be present.

The 2:4-dinitrophenylhydrazone was prepared from this ethylenic diurethane by the following procedure. 2:4-dinitrophenylhydrazine (200mg.) was dissolved in a warm mixture of acetic acid (5cc.) and water (1cc.). The ethylenic diurethane (200mg.) was added and the mixture was heated at 100° for one hour. Violet needles of the hydrazone crystallised out from the warm solution. Recrystallisation from aqueous acetic acid (90%) gave the phenylhydrazone as violet needles (100mg.), m.p. 174-175°. (Found : N, 19.87. $C_{11}H_{12}O_5N_4$ requires N, 19.99%)

An attempted partial reduction, as described by Campbell and Eby(32), of the acetylenic diurethane (XXI) using sodium alone in liquid ammonia gave only highly impure starting material ; the reaction time was four hours. When the reaction time was increased to eight hours and excess sodium used (total 6 mols.) two products were obtained. The desired trans-ethylenic diurethane already described above m.p. 101-102°, in 24% yield and a product crystallising as needles, m.p. 123-124°, from a light petroleum (b.p. 60-80°)-methanol mixture (1:4) in 20% yield. (Found : C, 50.2; H, 6.8; N, 13.3. $C_9H_{16}O_4N_2$ requires C, 50.0 ; H, 7.4 ; N, 13.0%). Quantitative microhydrogenation showed 0.95 double bonds present. This product was almost certainly trans 1:1-(dicarb-

methoxyamino)pent-3-ene, the excess sodium having caused hydrogenolysis of the allylic hydroxyl group. In order to confirm that this latter compound was not the fully saturated diurethane a sample of 1:1-(dicarbmethoxyamino)pent-3-yn-5-ol was hydrogenated over 10% palladium-charcoal catalyst whereby two moles of hydrogen were absorbed. The product was a liquid.

The 2:4-dinitrophenylhydrazone, prepared as described for the trans-ethylenic diurethane above, crystallised from acetic acid as orange needles, m.p. 189-190°. (Found : N, 20.3. $C_{11}H_{14}O_5N_4$ requires N, 19.9%).

Attempted Prevost reaction on trans 1:1-(dicarbmethoxyamino) pent-3-en-5-ol.- The method employed is that described by Wittcoff and Miller(33a).

Silver benzoate (0.458g.) was heated under reflux for 30 minutes with iodine (0.252g.) in sodium dried benzene (150cc.). The violet colour disappeared in 10 minutes and a bulky yellow precipitate appeared. The ethylenic diurethane (0.232g.) was added and the solution heated under reflux for 24 hours. After filtration the benzene was removed in vacuo leaving a residual syrup. On trituration with ethyl acetate crystallisation occurred, the solid product consisting of starting material (0.110g.). The residual glass obtained by evaporating off the ethyl acetate could not be induced to crystallise. An attempt to prepare the 2:4-dinitrophenyl-hydrazone as described for the ethylenic diurethane (XXll) gave no identifiable product.

1:1-(Dicarbmethoxyamino)3:4-epoxypentan-5-ol (XXIII).-
A standard solution of perbenzoic acid in chloroform was prepared as described by Braun(36). Trans 1:1-(dicarbmethoxyamino)pent-3-en-5-ol (XXII ; 0.5g.) was dissolved in chloroform (4cc.) and the solution cooled to 0°. Perbenzoic acid in chloroform (6.65cc.; 4.45% w/v.; 1 mol.) was added and the solution kept at 0° for four hours and then at 20° for twenty hours. The chloroform was removed in vacuo and the residue crystallised from ethyl acetate giving 1:1-(dicarbmethoxyamino)3:4-epoxypentan-5-ol as prisms (0.155g.; 29%), m.p. 122°. (Found : C, 43.73 ; H, 6.47 ; N, 11.40. $C_9H_{16}O_6N_2$ requires C, 43.54 ; H, 6.50 ; N, 11.29%)

Attempted hydrolysis of 1:1-(dicarbmethoxyamino)3:4-epoxypentan-5-ol (XXIII).-
(A) The epoxide (100mg.) was dissolved in water (3cc.) and the solution heated at 90° for three hours. The solution quickly turned yellow and became light brown in the course of three hours. On removal of the water under reduced pressure a gummy yellow glass remained. The glass could not be induced to crystallise and with Dische reagent gave a violet colour which changed to green on standing. On being heated further at 100° with dilute formic acid (15cc.; 4%) followed by the removal of the solvent in vacuo the gummy glass appeared to be unchanged giving a violet colour turning green on standing with Dische reagent. 1:1'-Benzylphenylhydrazine

(50mg.) and acetic acid (0.5cc.) was added and the mixture allowed to stand two days in a vacuum desiccator over silica gel and solid sodium hydroxide. No benzylphenylhydrazone could be isolated.

(B) The epoxide (200mg.) was dissolved in dilute sulphuric acid (10cc.; 0.01N) and the solution heated at 90° for 15 minutes. The sulphate ion was removed by the anion exchange resin Amberlite IR-4B; removal of water under reduced pressure yielded a yellow glass which gave with Dische reagent a dull red colour turning green on standing. p-Nitrophenylhydrazine (100mg.) and ethanol (10cc.) was added to the crude product and the solution heated under reflux for four hours. The red oil which remained after removal of the solvent was chromatographed on alumina using methanol as a developer. p-Nitrophenylhydrazine was recovered as a rapidly moving yellow band. The top yellow band was eluted with boiling methanol and on removal of the solvent gave only a high melting (over 300°) amorphous mass.

(C) The epoxide (200mg.) was dissolved in dilute sulphuric acid (50cc.; 0.02N) and allowed to stand 22 hours at 20°, then heated 15 minutes at 60°. The solution, when cool, was extracted with ether (10 × 50cc.) and the sulphate ion removed with Amberlite resin IR-4B. The water was then removed *in vacuo* and the resulting glass allowed to stand 24 hours over

phosphorous pentoxide. On trituration with dry ether followed by iso-propyl alcohol, a small quantity of an amorphous solid (m.p. over 360°) was filtered off. The residual glass (0.85g.) was dissolved in dry ethanol (4cc.) and freshly distilled aniline (0.14cc.) added. The solution was heated under reflux for six hours and the ethanol removed under reduced pressure. No solid anilide could be recovered from the residue.

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