SYNTHESES EMPLOYING ACETYLENIC COMPOUNDS

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THESIS

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C O N T E N T S

SUMMARY

Many of the varied reactions of acetylenic compounds have been well established for a number of years, and in recent times their utilisation in solving problems of organic synthesis has attracted attention. Successful and elegant work has resulted in many fields. In the present research, the application of the peculiar properties of the triple bond has been sought in the synthesis of compounds in the field of carbohydrates, including two natural products.

A meso-dideoxyhexitol, erythro-hexane-1:3:4:6-tetrol, has been synthesised from acetylene by an unambiguous method, and was not identical with the compound to which this constitution has hitherto been ascribed. The latter compound had arisen from an unexpected variant of the standard reaction, carbonyl reduction by Raney nickel hydrogenolysis of the acetylated sugar thioacetal, when applied to keto-D-psicose penta-acetate.

Two unusual pentoses have been synthesised. These compounds, apiose and cordycepose, are members of the small group of natural sugars having a branched carbon chain. Several promising synthetic approaches to apiose and cordycepose, some of which were based on

acetylenic intermediates, have been investigated and found to be impracticable. In the case of each sugar, the route that was eventually developed into a successful and smooth synthesis starts from the condensation of bromoacetal and ethyl malonate. The structures indicated by degradative evidence for apiose and cordycepose are thereby confirmed.

PART ONE

The Synthesis of erythro-Hexane-1:3:4:6-tetrol

PART ONE. The Synthesis of erythro-Hexane-1:3:4:6-tetrol

HISTORICAL INTRODUCTION

The growth of acetylene chemistry over the years has been followed by two developments in recent times, the discovery and elucidation of the structures of naturally occurring acetylenes and the widespread use of acetylenic intermediates in the synthesis of natural products. Both developments have in their turn expanded greatly the range and depth of knowledge of acetylenic reactions. The versatility of the triple bond in synthesis results from its extreme but well-controllable reactivity, particularly towards nucleophilic reagents. Furthermore acetylene and monosubstituted acetylenes form metallic derivatives which can be used as synthetic reagents in the same way as the familiar Grignard compounds.

There exist now several examples of the application of acetylenic intermediates to synthesis in the carbohydrate field, and the key reactions are by now well established. So far the end-products aimed at have been mostly sugar alcohols.

The pioneer in this use of acetylenic compounds was

Lespieau and the results of his work during the years 1928-1938 are collected, together with the numerous relevant references, in his review in "Advances in Carbohydrate Chemistry" (1). The history of the acetylenic approach is preceded by two records of the use of ethylenic starting materials, the first by Griner (1892) who converted into mannitol one of two stereoisomers (I, see flowsheet on p. 13) produced by the addition of hypochlorous acid to divinylglycol (II), the latter being obtained by bimolecular reduction of acrolein. Later Lespieau and Wiemann (1933) improved Griner's route by using the meso-form of divinylglycol to obtain the previously unknown hexitol allitol (III), as well as the DL-form to obtain DL-mannitol. This synthesis had one step fewer, as the divinyl glycol was hydroxylated directly with silver chlorate-osmium tetroxide.

Flexibility was gained by the adoption of the acetylenic route, but its first illustrations suffered from excessive length. Lespieau's synthesis of allitol and dulcitol is shown on the flowsheet on p. 14. Chloroacetaldehyde and acetylenedimagnesium bromide were condensed to give principally the meso-form of the acetylenic glycol (IV). Treatment of (IV) with potassium hydroxide, hydrolysis of the diepoxide (V), and then partial hydrolysis with a palladium catalyst yielded the cis-ethylene (VI).

Hydroxylation of (VI) with silver chlorate-osmium tetroxide (cis-hydroxylation, see p. 11) gave mainly allitol, whereas hydroxylation of the tetra-acetate of (VI) with the same reagent gave, after hydrolysis, mainly dulcitol.

An extension of this hexitol synthesis was demonstrated by Wiemann (1936), who prepared the decanehexol (VII).

$$CH_3 \cdot CH_2 \cdot (CHOH)_6 \cdot CH_2 \cdot CH_3 \qquad (VII)$$

The first synthesis of pentitols (also by Lespieau, in 1936-1938) is closely analogous to the hexitol route. The starting aldehyde in this case is acrolein dichloride and similar reactions were carried through to derive the chloroacetylenic diol (VIII, p. 15); conversion into the triacetate (IX) was followed by hydrogenation, hydroxylation, and acetylation to give two isomeric penta-acetates (X), hydrolysis of which after separation gave ribitol and DL-arabitol. No xylitol could be detected as a product of this sequence and Lespieau therefore concluded that the diol (VIII), a pure racemate, had the erythro-configuration.

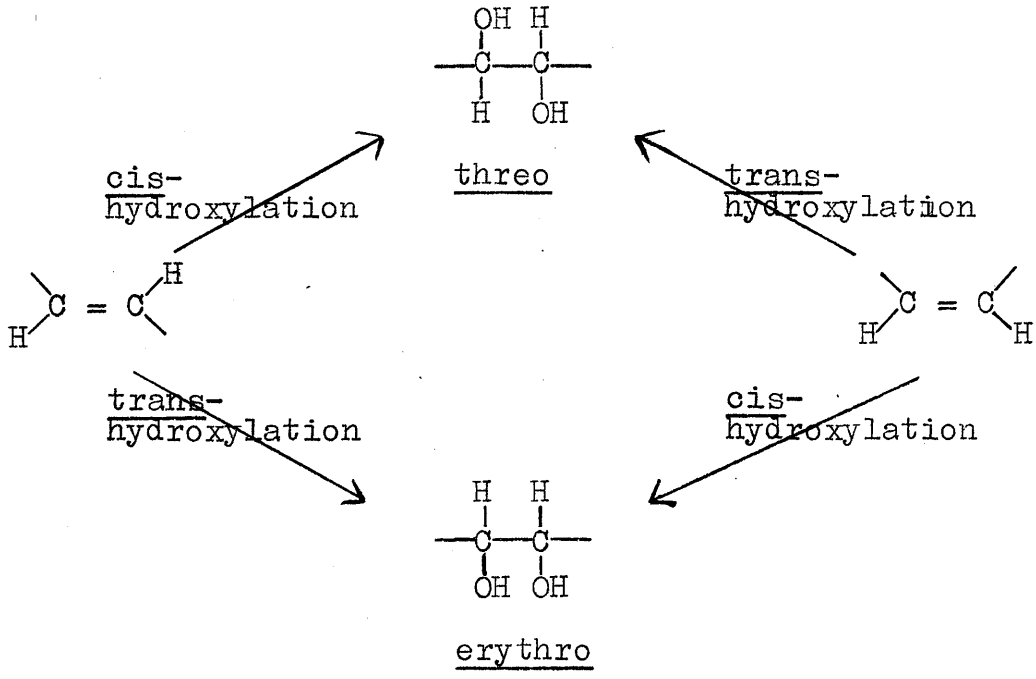
The most important reactions of acetylenic compounds germane to carbohydrate synthesis are firstly the convenient preparation by standard methods of acetylenic

carbinols and glycols from the metal derivatives of acetylene or monoalkylacetylenes by reaction with carbonyl compounds (or ethylene oxides); secondly, the possibility of effecting exclusive addition to either the double or triple bond where both are present in a molecule; thirdly, stereospecific addition of hydrogen to an acetylene to give the corresponding cis- or trans-ethylene, the mode of addition being determined by the reagent; and fourthly, the stereospecific hydroxylation of the ethylenes thus produced. It can be shown theoretically and is amply confirmed in practice that cis-addition to a cis-double bond or trans-addition to a trans-double bond yields the corresponding erythro-compound, and that cis-addition to a trans-double bond or trans-addition to a cis-double bond yields the threo-derivative. The preparation of vicinal dihydroxy-compounds from a triply-bonded precursor is thus attended by complete steric control at every stage.

Partial reduction of a triple bond may be effected either by catalytic hydrogenation or by use of a "chemical" reducing agent. The most convenient of the latter is sodium in liquid ammonia, which affords exclusively the trans-ethylene (2). Depending on both the catalyst and the acetylene, the hydrogenation process may or may not be subject to stoppage or deceleration when one mol. of hydrogen has been absorbed; but usually a good

yield of the ethylene can be obtained by stopping the absorption at that point. Palladium catalysts are most often used and the overwhelming product is the cis-ethylene (3).

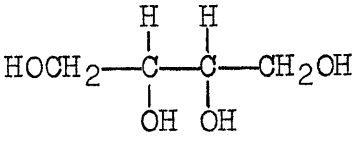
Reagents which have been shown to effect cis-hydroxylation of ethylenes include osmium tetroxide (4)(5), hydrogen peroxide catalysed by osmium tetroxide (6)(7), metal chlorates catalysed by osmium tetroxide (8), silver acetate-iodine in wet acetic acid (9), and alkaline permanganate (4)(10)(11)(12)(13). trans-Addition results when hypohalous acids (14), per-acids followed by fission of the epoxide ring so produced (14), in simple cases hydrogen peroxide catalysed by tungsten trioxide (15), or the iodine-silver benzoate complex (16) are used.



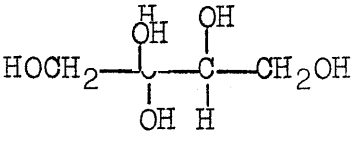
Essentially the same route as Lespieau's —— but

much shortened —— has been followed in a more recent synthesis of ribitol and DL-arabitol (17). The readily obtainable trans-pent-2-en-4-yn-1-ol (XI; flowsheet, p. 16) (18) was treated with performic acid and the resulting monoformate was hydrolysed by steam distillation; the triple bond is unaffected by this reagent and DL-pent-4-yn-1:2:3-triol (XII) was obtained. Degradation, by ozonolysis to DL-erythronic acid and by oxidation with nitric acid to meso-tartaric acid, showed that (XII) had the expected erythro-configuration, brought about by trans-hydroxylation of a trans-double bond. (XII) was acetylated to give the triacetate (IX), which Lespieau had obtained (see p. 9). Thence partial catalytic hydrogenation and treatment with N-bromosuccinimide in water, which acted as a source of hypobromous acid, gave two isomeric bromohydrins (XIII); separation followed by acetylation and hydrolysis gave ribitol and DL-arabitol.

The synthesis of the two tetritols, erythritol (XIV) and DL-threitol (XV) is achieved simply from the



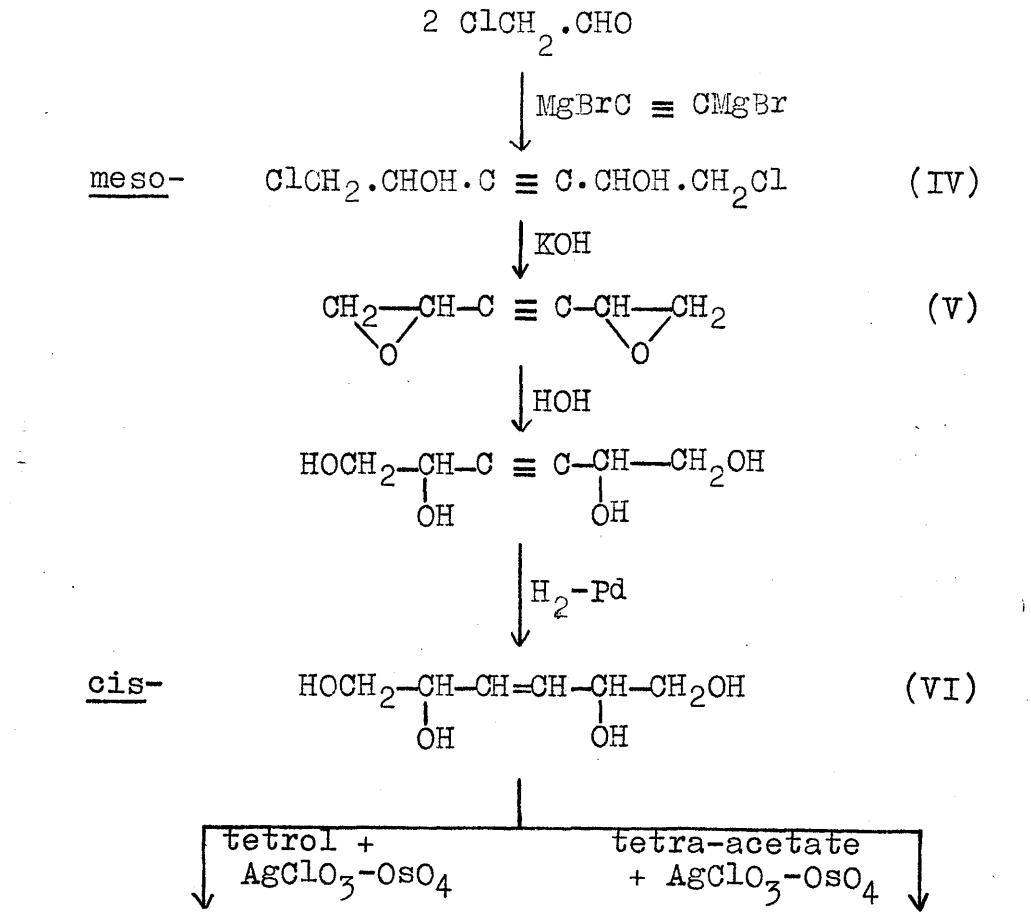
(XIV)

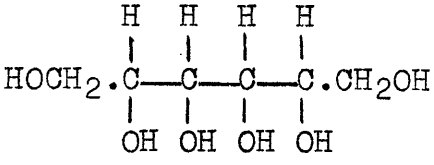


(XV)

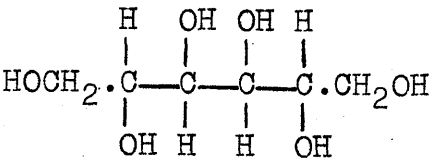
readily available but-2-yne-1:4-diol by semihydrogenation

Hexitol synthesis



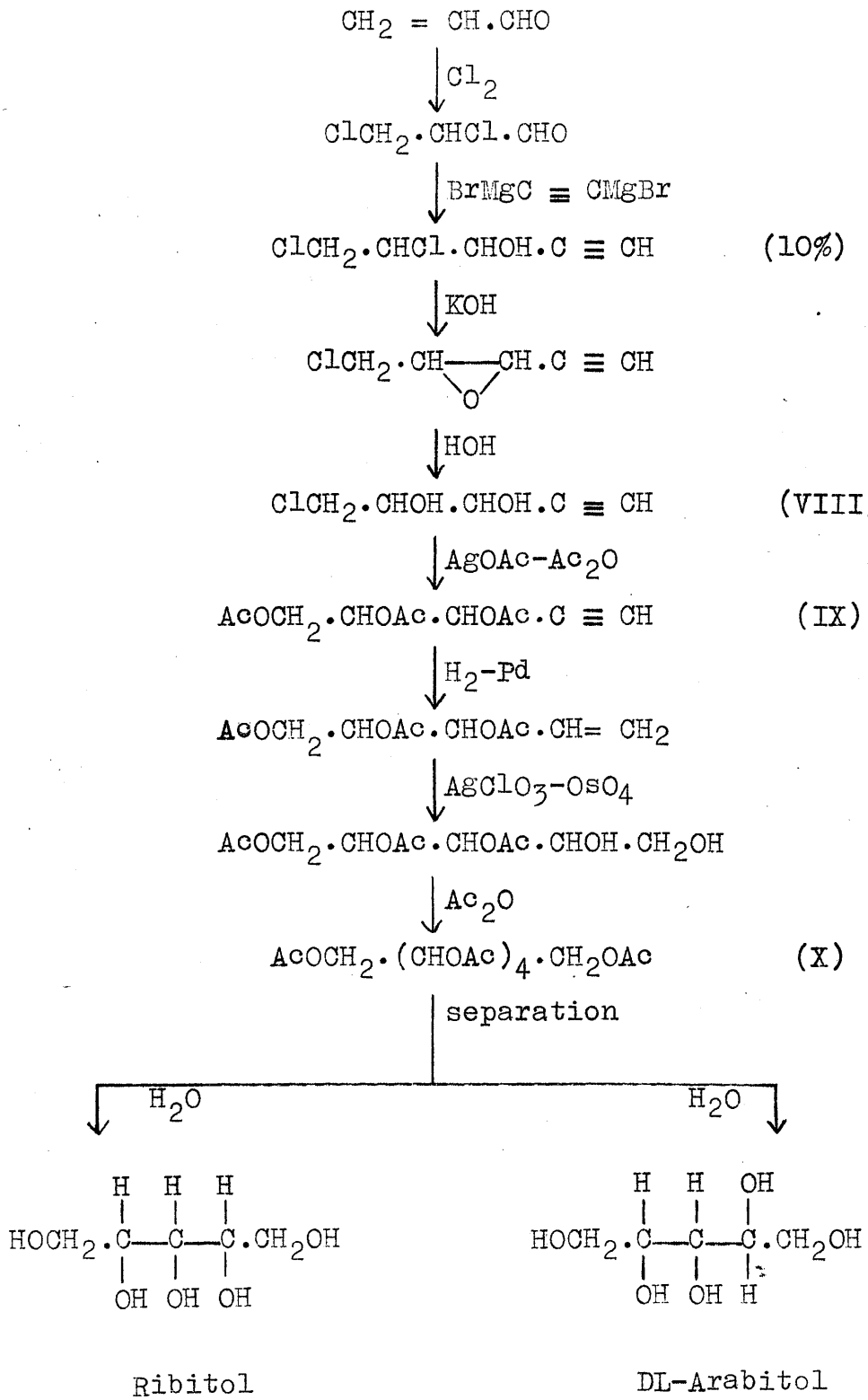


Allitol (III)



Dulcitol

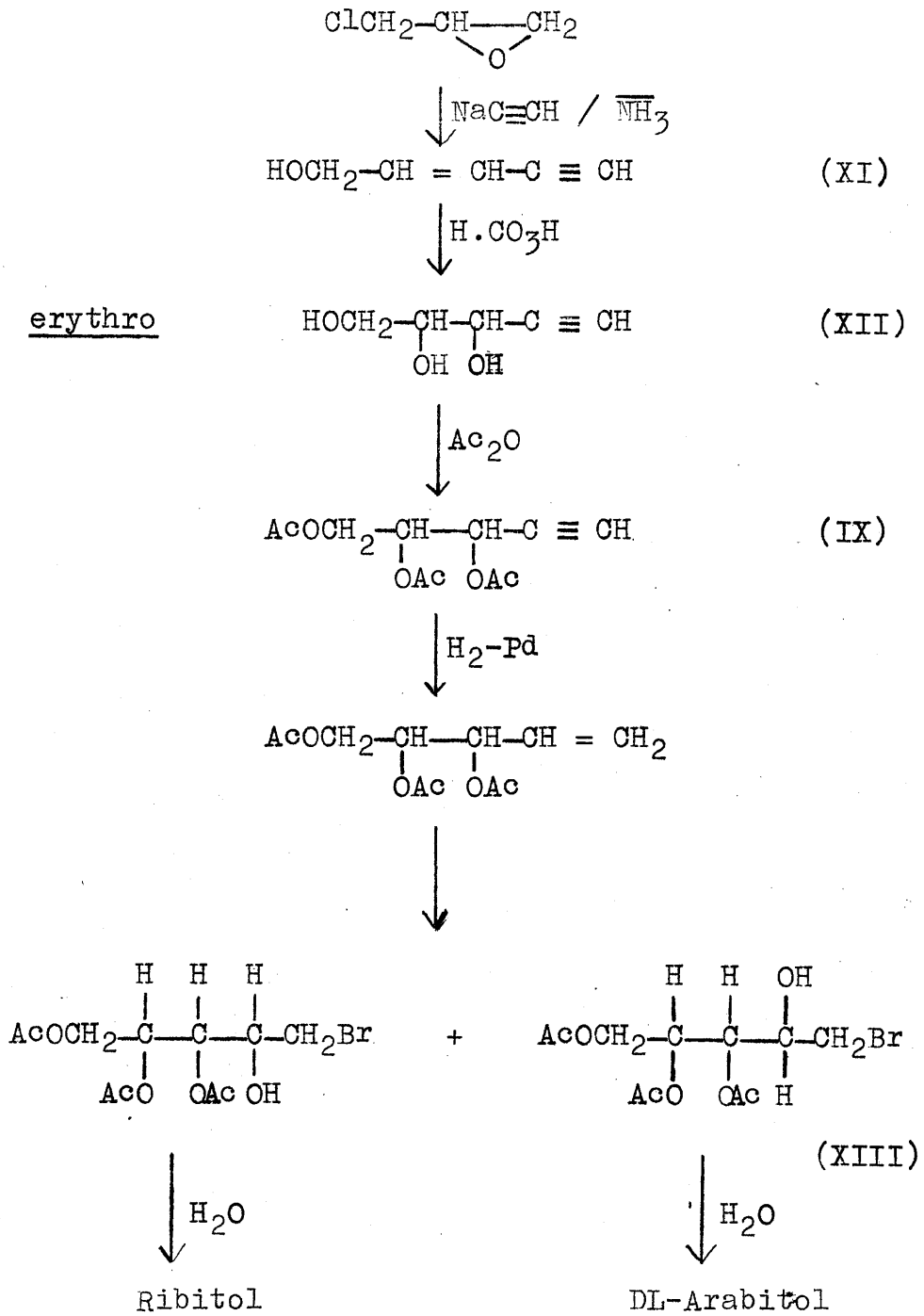
Pentitol synthesis



Ribitol

DL-Arabitol

Pentitol synthesis

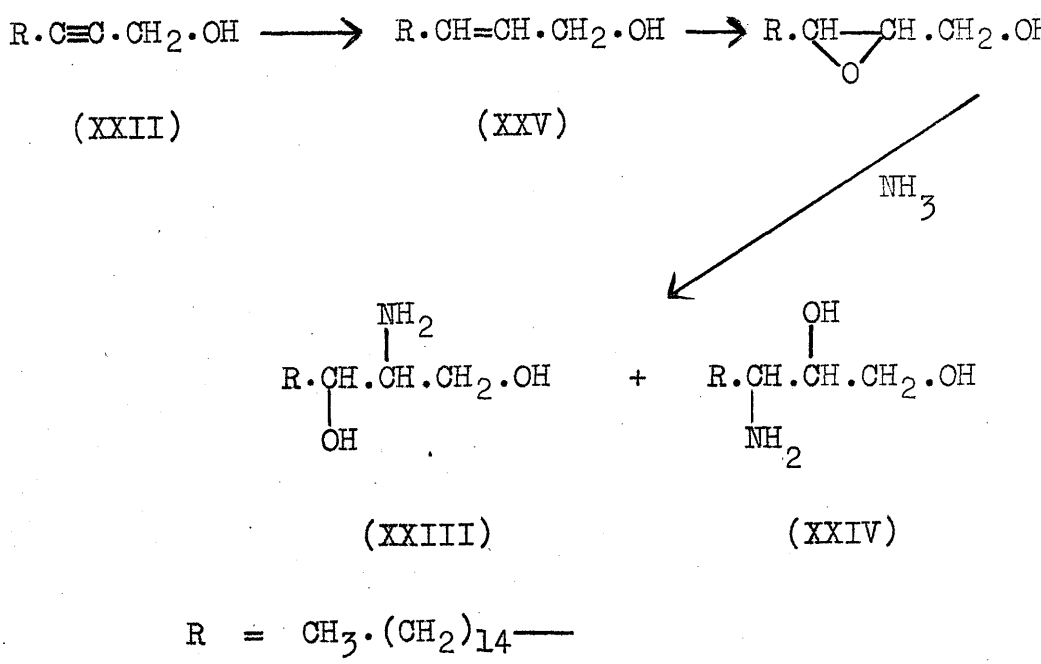


to the cis- or trans-but-2-ene-1:4-diol, followed by hydroxylation of the appropriate isomer (19).

The biochemically important deoxypentose, 2-deoxyribose, has been synthesised from an acetylenic precursor (20). Interest here lies in the method of synthesis, rather than in the preparative value, as the yield was low. But-2-yne-1:4-diol was converted into a monohalogeno-derivative by treatment of the half-benzoate with phosphorus tribromide to give 1-benzoyloxy-4-bromo-but-2-yne (XVI; flowsheet, p. 19). Condensation of (XVI) with ethyl sodiomalonate gave the ester (XVII), which was treated with hydrazine to form the dihydrazide (XVIII). A double Curtius rearrangement of (XVIII) produced the acetylenic diurethane (XIX); thereafter partial catalytic hydrogenation to the corresponding cis-ethylene followed by cis-hydroxylation gave the erythro-triol (XX). Careful hydrolysis of (XX) gave a small yield of 2-deoxy-DL-ribose.

Another recent application of these techniques has been the synthesis of threo-dihydrosphingosine (XXI, p. 18) from the acetylenic alcohol (XXII)(21). Partial catalytic hydrogenation, treatment with perphthalic acid, and then ring fission by ammonia gave the two threo-dihydroxyamines (XXIII) and (XXIV); the action of periodic acid on the mixture of N-acetyl derivatives destroyed the

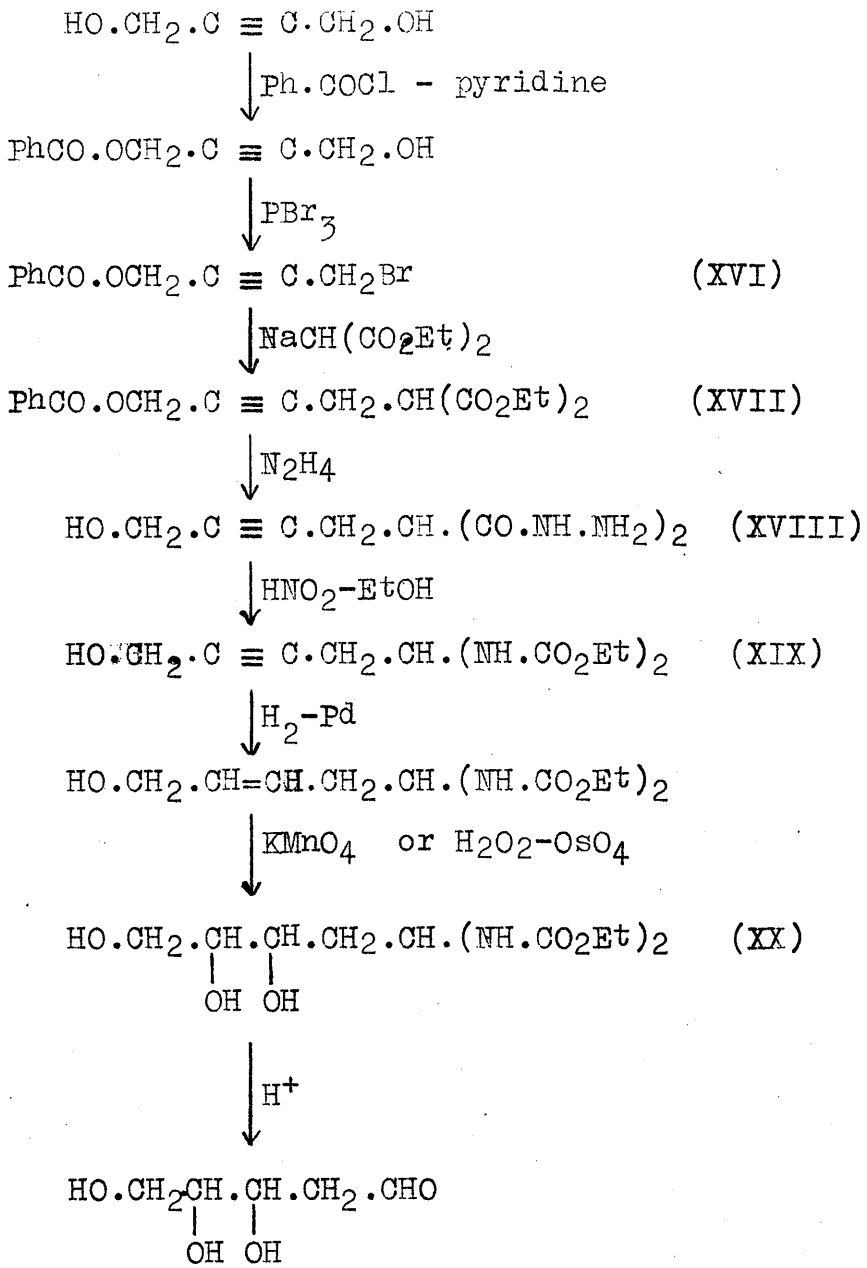
unwanted isomer (XXIV). erythro-Dihydrosphingosine was obtained similarly by commencing with reduction of the acetylenic alcohol (XXII) to the trans-ethylenic alcohol (XXV)(22).



(XXII) (XXV)

(XXIII) (XXIV)

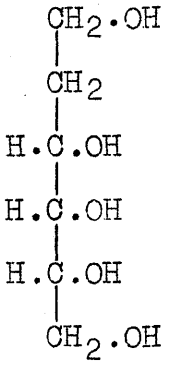
R = $CH_3.(CH_2)_{14}$—



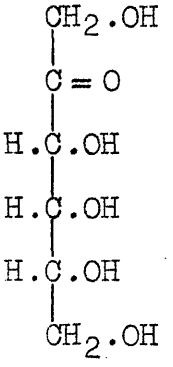
2-Deoxy-DL-ribose

THEORETICAL

In order to prove that a 2-deoxyhexitol produced by the electrolytic reduction of D-glucose under alkaline conditions was (as theory predicted) 2-deoxy-D-allitol (XXVI), Wolfrom, Lew, and Goepp (23) in 1946 attempted the preparation of the latter, a hitherto unknown compound. The method chosen was the reduction of the corresponding ketose having the same configuration, namely keto-D-psicose (XXVII), via the Raney nickel hydrogenolysis of its thioketal penta-acetate —— a sequence which had already successfully converted keto-D-fructose penta-acetate into 2-deoxysorbitol penta-acetate (24).



(XXVI)

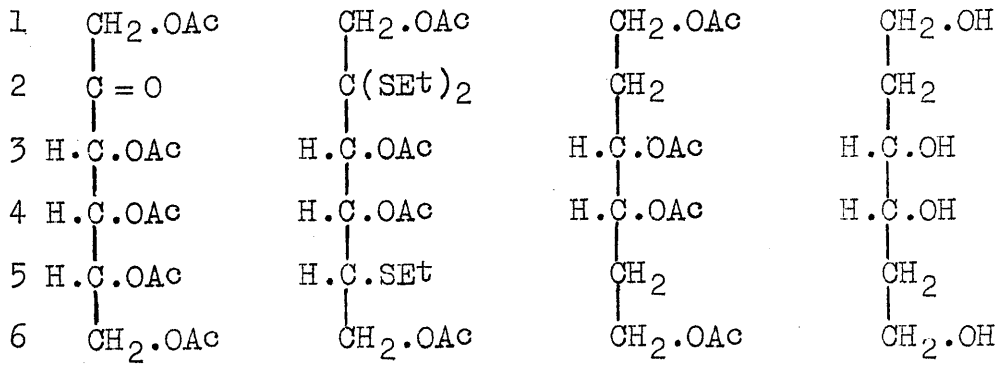


(XXVII)

Crystalline keto-D-psicose penta-acetate was prepared (25) and was treated with ethanethiol and zinc chloride to

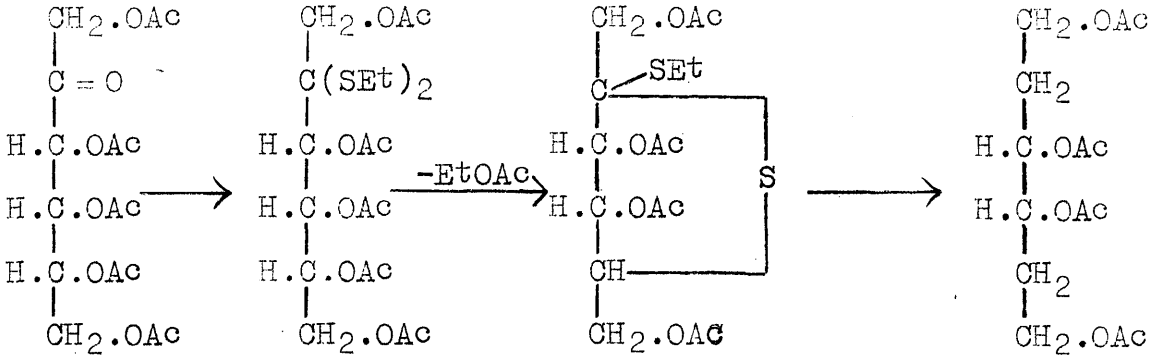
convert it into the corresponding thioketal (26); the latter was then subjected to Raney nickel hydrogenolysis. The product of each step was a crude syrup. After deacetylation with barium hydroxide, a pure crystalline polyhydroxy compound was isolated, and was characterised as the dimethylene derivative. Most unexpectedly it had the composition of a dideoxyhexitol.

Since an aqueous solution of the compound was devoid of optical activity throughout the visible spectrum, Wolfrom and his co-workers concluded that it had the meso-structure erythro-hexane-1:3:4:6-tetrol (XXVIII). To interpret this surprising result they proposed the following mechanism to account for the replacement of the 5-acetoxy group:



(XXVIII)

Hydrolysis of the 5-acetoxy group before replacement was considered to be a further possibility. In the author's opinion the following mechanism would be considerably more plausible:



Although some support for such a replacement is afforded in the literature, the unusual nature of the postulated reaction prompted the planning of an unequivocal synthesis of erythro-hexane-1:3:4:6-tetrol, for purposes of comparison with the product.

The key compound in the synthesis of this structure by means of an acetylenic precursor is hex-3-yne-1:6-diol (XXIX) flowsheet on p. 27) which lends itself to the sequence of stereospecific reactions of hydrogenation and hydroxylation discussed on p. 10. The direct one-stage synthesis of this diol from acetylene, namely the reaction of acetylenedimagnesium bromide with ethylene oxide, was unsuccessful; ethylene bromohydrin alone was isolated in quantity. A review of the Grignard reaction by Gaylord and Becker (27) includes previously reported examples of this result of the interaction of ethylene oxide and Grignard reagents. To avoid the Grignard type of reaction therefore, which would allow both hydroxyl

groups to be introduced into the molecule simultaneously, the alkali metal acetylide technique was resorted to. This necessitated a two-step procedure as shown on p. 27.

The starting material was but-3-yn-1-ol, the product of interaction of sodium acetylide and ethylene oxide. The hydroxyl group of this compound was protected by treatment with 2:3-dihydropyran. The resulting 4-(tetrahydro-2-pyranyloxy)but-1-yne (XXX)(28) was converted into its lithium salt by reaction with lithamide in liquid ammonia; reaction with ethylene oxide then produced 6-(tetrahydro-2-pyranyloxy)hex-3-yn-1-ol (XXXI). Use of the corresponding sodium compound was found to give a poorer yield. For proof of skeletal structure the compound (XXXI) was completely hydrogenated catalytically and the resulting saturated compound hydrolysed with methanol—hydrochloric acid to the known hexane-1:6-diol. Smooth acid hydrolysis of the acetal linkage in (XXXI) could not be obtained by aqueous sulphuric, oxalic, or hydrochloric acids. Such failure is hard to understand. Not only did all experiments yield a mixture of liquids, but under moderate conditions a large part of the material was returned unchanged. Repeated treatment augmented the product, as did use of 2N hydrochloric acid rather than oxalic acid or sulphuric acid, but not sufficiently. A possible complication in the reaction is hydration of the triple bond,

and although there were indications of this, its occurrence was not proved. By contrast a most successful reagent was found to be dry methanolic sulphuric acid and this method afforded crystalline hex-3-yne-1:6-diol in satisfactory yield. A bisphenylurethane was formed and complete hydrogenation gave hexane-1:6-diol. The overall yield of hex-3-yne-1:6-diol from but-3-yn-1-ol was 46%.

While the hydrolysis of the ether (XXXI) was requiring so much troublesome investigation, a variation of the synthetic route was explored, by which removal of the tetrahydropyranyl group was delayed to a later and possibly final stage. 6-(Tetrahydro-2-pyranyloxy)hex-3-yn-1-ol was partially hydrogenated with palladised charcoal as catalyst. The cis-ethylene which resulted was hydroxylated with a tert.-butanol solution of hydrogen peroxide containing osmium tetroxide, and the syrupy product was acetylated as a preliminary to hydrolysis of the blocking group. The product was however an inhomogeneous liquid and with the success of the methanolysis of 6-(tetrahydro-2-pyranyloxy)hex-3-yn-1-ol achieved by this time, the modified route was not further studied.

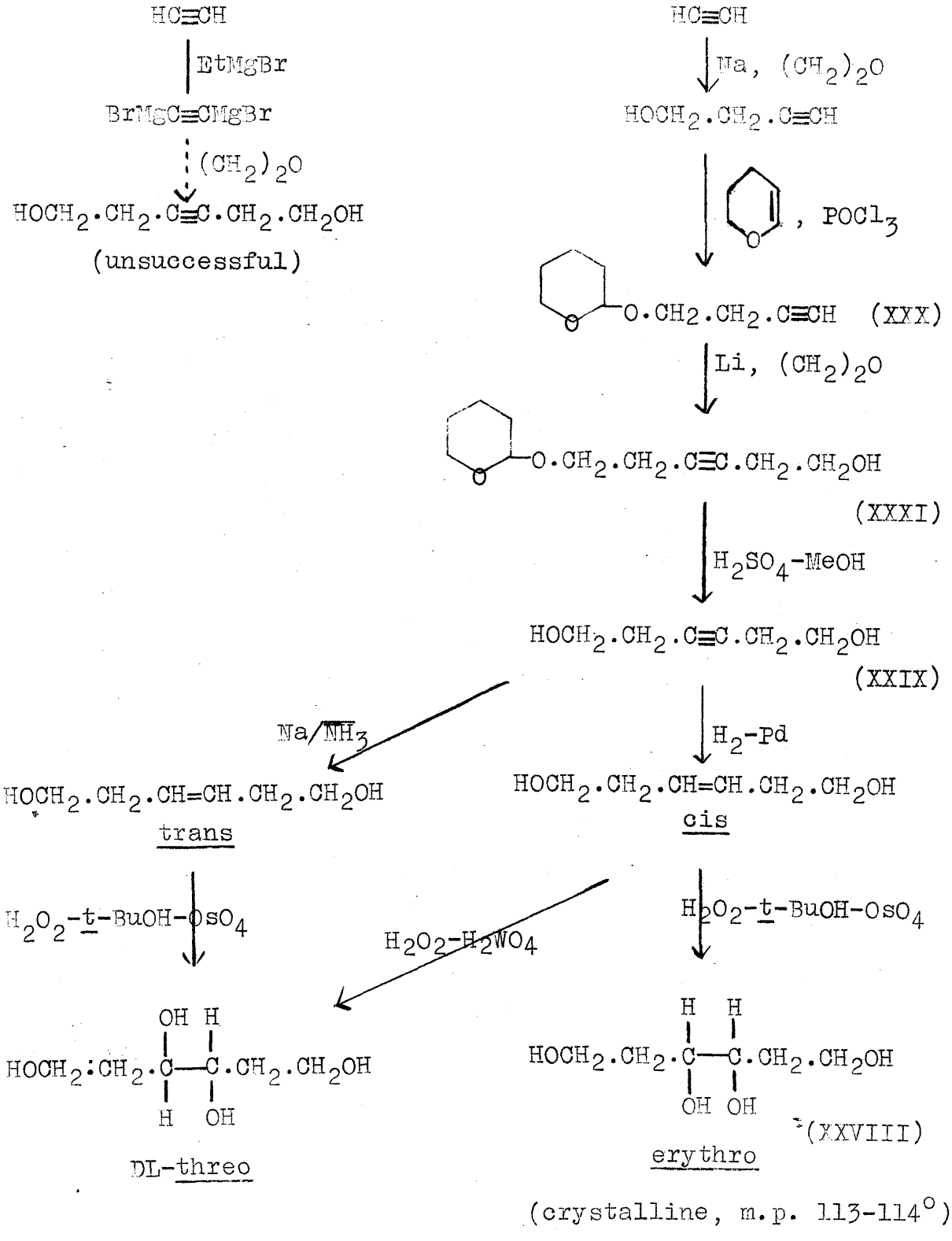
cis- and trans-Hex-3-ene-1:6-diols were each prepared from hex-3-yne-1:6-diol by means of the appropriate reducing agents, hydrogen in the presence of palladium and

sodium in liquid ammonia respectively. Both hexenediols were liquids sufficiently hygroscopic to defeat repeated attempts at accurate elementary analysis but each was characterised by its bisphenylurethane.

Treatment of the cis-diol with a tert.-butanolic solution of hydrogen peroxide containing osmium tetroxide (6) resulted in cis-hydroxylation to furnish erythro-hexane-1:3:4:6-tetrol (XXVIII) as a crystalline solid; the threo-hexane-1:3:4:6-tetrol obtained by similar treatment of the trans-diol was a syrup. A syrup was also obtained by the action (trans-hydroxylation, cf. ref. 15) of hydrogen peroxide catalysed by pertungstic acid on the cis-diol. Both the erythro-tetrol and the threo-tetrol were subjected to the procedure for formalation described by Wolfrom, Lew, and Goepp (23) to obtain the dimethylene derivative of each. The derivative of the erythro-tetrol was isolated in crystalline form, but the specimens of the threo-tetrol derived from both the above-mentioned routes yielded an oil which has failed to crystallise.

The m.p. of erythro-hexane-1:3:4:6-tetrol (113-114°) and that of its dimethylene derivative (146-147°) showed it to differ from the compound (m.p. 121-122°; dimethylene derivative, m.p. 97-98°) obtained by the American workers from the reaction sequence starting

from keto-D-psicose penta-acetate. The compound has therefore not the constitution they assigned to it and an alternative explanation of the route of the desulphurisation reaction becomes necessary.


HC≡CH
EtMgBr
BrMgC≡CMgBr
(CH2)2O
HOCH2.CH2.C≡C.CH2.CH2OH
(unsuccessful)
HC≡CH
Na, (CH2)2O
HOCH2.CH2.C≡CH
, POCl3
O.CH2.CH2.C≡CH (XXX)
Li, (CH2)2O
O.CH2.CH2.C≡C.CH2.CH2OH
(XXXI)
H2SO4-MeOH
HOCH2.CH2.C≡C.CH2.CH2OH
(XXIX)
Na/NH3
H2-Pd
HOCH2.CH2.CH=CH.CH2.CH2OH
trans
HOCH2.CH2.CH=CH.CH2.CH2OH
cis
H2O2-t-BuOH-OsO4
H2O2-H2WO4
H2O2-t-BuOH-OsO4
OH H
HOCH2.CH2.C—C.CH2.CH2OH
H OH
DL-threo
H H
HOCH2.CH2.C—C.CH2.CH2OH
OH OH
(XXVIII)
erythro
(crystalline, m.p. 113-114°)

EXPERIMENTAL

Attempted direct preparation of hex-3-yne-1:6-diol from acetylene.

A solution of ethylmagnesium bromide (from magnesium, 12 g.) was prepared in the usual way. By the addition of hot dry benzene (450 c.c.) to the solution on the water-bath, followed by distillation of ether, most of the ether was replaced by benzene, in order that the acetylenedimagnesium bromide to be produced should separate in a solid rather than a gummy form. Pure, dry acetylene was passed into the solution in benzene, with stirring, for 24 hours and then ethylene oxide (44 g.) in cold dry benzene was added in 20 minutes with stirring and cooling. Stirring was continued for a further 4½ hours, after which time the mixture was left overnight and then poured into dilute sulphuric acid containing crushed ice. The benzene layer was separated and the aqueous layer (containing an excess of sodium sulphate) was extracted with ether for 12 hours in a continuous liquid extraction apparatus. The combined benzene-ether extract was washed with concentrated sodium sulphate solution and dried ($MgSO_4$). Distillation of the liquid (45 g.) following removal of solvent gave predominantly ethylene bromohydrin together with smaller amounts of other volatile products, very probably including

but-3-yn-1-ol. The quantity of higher-boiling material available for working up a glycol product was negligible.

4-(Tetrahydro-2-pyranyloxy)but-1-yne.

This compound was prepared in 85% yield by keeping a mixture of but-3-yn-1-ol and 2:3-dihydropyran for 2 hours with a catalytic quantity of phosphorus oxychloride, according to the method of Henbest, Jones, and Walls (29). The product was a liquid, b.p. 86-87°/13 mm., n_D^{23} 1.4580. (Jones, Shen, and Whiting, ref. 28, give b.p. 92-95°/18 mm., n_D^{18} 1.4589.)

6-(Tetrahydro-2-pyranyloxy)hex-3-yn-1-ol.

To a stirred solution of lithamide (from lithium, 2.3 g., in the presence of ferric nitrate catalyst) in liquid ammonia (400 c.c.) was added dropwise (15 minutes) a solution of 4-(tetrahydro-2-pyranyloxy)but-1-yne (45.4 g.) in dry ether (25 c.c.), and stirring was continued for 40 minutes. Ethylene oxide (40 c.c.) was then added all at once and the reaction mixture was stirred for 9 hours, after which it was decomposed by addition of ammonia solution (d 0.88; 5 c.c.) and set aside overnight for the ammonia to evaporate. Ether and water were added, the aqueous layer was extracted with ether, and the ethereal solution was washed with brine, dried (Na_2SO_4), and

evaporated. Distillation gave 6-(tetrahydro-2-pyranyloxy)hex-3-yn-1-ol (45 g., 77%) as a liquid, b.p. 116°/0.4 mm., n_D^{21} 1.4828, after a small fore-run (5.5 g.) of starting material (Found: C, 66.3; H, 9.3. $C_{11}H_{18}O_3$ requires C, 66.6; H, 9.15%).

Catalytic hydrogenation of the compound in ethyl acetate in the presence of platinic oxide resulted in the uptake of 2 mols. of hydrogen, to form 6-(tetrahydro-2-pyranyloxy)hexan-1-ol, b. p. 97°/0.5 mm., n_D^{21} 1.4570, the phenylurethane of which crystallised in plates, m.p. 73-74°, from light petroleum (b.p. 40-60°)(Found: N, 4.6. $C_{18}H_{27}O_4N$ requires N, 4.4%).

The product of hydrogenation (0.7 g.) was converted into hexane-1:6-diol by shaking for 2 days at room temperature with concentrated hydrochloric acid (1 c.c.), methanol (1 c.c.), and ether (20 c.c.). The product, isolated by evaporation followed by drying over potassium hydroxide in a vacuum desiccator and distillation, crystallised completely on further drying in needles, m.p. and mixed m.p. with hexane-1:6-diol 40-41°.

Hydrolysis of 6-(tetrahydro-2-pyranyloxy)hexan-1-ol by hydrochloric acid (2N) was found to be unsatisfactory.

Hex-3-yne-1:6-diol.

6-(Tetrahydro-2-pyranyloxy)hex-3-yn-1-ol (5 g.) was added to a solution of concentrated sulphuric acid

(4 c.c.) in methanol (90 c.c.) and set aside at room temperature for 48 hours. The mixture was then neutralised with methanolic sodium methoxide (phenolphthalein as indicator), the solution being kept cooled to room temperature. The methanol was evaporated under reduced pressure — an efficient splash-head was necessary — and the gelatinous, colourless, mainly solid residue was extracted with hot ethyl acetate (3 x 50 c.c.). The filtered extract was taken to dryness and the residue dissolved in boiling benzene (50 c.c.). Filtration and cooling gave elongated plates (2 g., 70%) of hex-3-yne-1:6-diol, m.p. 77-79°; recrystallisation from benzene-light petroleum (b.p. 60-80°) gave the pure diol, m.p. 80-80.5° (Found: C, 63.25; H, 8.85. $C_6H_{10}O_2$ requires C, 63.15; H, 8.85%). This procedure for neutralisation was found preferable to a method employing stirring with barium carbonate and dry ether followed by filtration of the resulting barium sulphate.

Setting aside a concentrated solution of the diol (100 mg.) and phenyl isocyanate (0.5 c.c.) in dioxan at room temperature for several days gave the bisphenylurethane, which crystallised from benzene, followed by ethanol, in plates, m.p. 173-174° (Found: C, 68.4; H, 5.6; N, 8.3. $C_{20}H_{20}O_4N_2$ requires C, 68.2; H, 5.7; N, 7.95%). Complete hydrogenation catalysed by platinic oxide in ethyl acetate

resulted in the uptake of 2 mols. of hydrogen to furnish hexane-1:6-diol, m.p. and mixed m.p. 39-41°.

Attempted hydrolysis of 6-(tetrahydro-2-pyranyloxy)hex-3-yn-1-ol, by aqueous acid.

The following are examples of the conditions used in the experiments. None of the resulting fractions could be crystallised.

(a) 6-(Tetrahydro-2-pyranyloxy)hex-3-yn-1-ol (7.5 g.) was shaken for 1½ hours with sulphuric acid (5N; 25 c.c.). Sodium chloride was added to salt out the product, which was extracted with ether. The extract was washed with a solution of sodium hydrogen carbonate and sodium chloride, then washed with brine, dried, and evaporated. The principal fraction obtained on distillation consisted of unchanged starting compound, b.p. ca. 100°/0.3 mm., n_D^{20} 1.482. Retreatment with heating to 100° had a similar result, a rather higher proportion of the material undergoing reaction.

(b) 6-(Tetrahydro-2-pyranyloxy)hex-3-yn-1-ol (3 g.) was heated with oxalic acid (1 g.) in water (10 c.c.) at the boiling point for 1 hour. The mixture was worked up as in (a) with substantially the same result.

(c) 6-(Tetrahydro-2-pyranyloxy)hex-3-yn-1-ol (1 g.) was shaken for 30 minutes with hydrochloric acid (2N; 5 c.c.). The mixture was neutralised with sodium carbonate and then evaporated to dryness, and the product was taken up in ethyl acetate. Among the fractions obtained on distillation was

a liquid (0.3 g.), b.p. *ca.* 80°/0.2 mm., n_D^{25} 1.4608, which on catalytic hydrogenation absorbed only 60% of the volume of hydrogen theoretically required by hex-3-yne-1:6-diol.

cis-6-(Tetrahydro-2-pyranyloxy)hex-3-en-1-ol.

6-(Tetrahydro-2-pyranyloxy)hex-3-yn-1-ol (5.0 g.) in ethyl acetate was hydrogenated in the presence of palladium-charcoal (10%; 0.5 g.). The uptake of hydrogen was stopped when 1 mol. had been absorbed. After removal of catalyst and solvent, fractional distillation yielded *cis*-6-(tetrahydro-2-pyranyloxy)hex-3-en-1-ol (4.1g.) as a liquid, b.p. 100-103°/0.3 mm., n_D^{25} 1.4719.

Hydroxylation of *cis*-6-(tetrahydro-2-pyranyloxy)hex-3-en-1-ol.

A cooled mixture of the *cis*-ethylenic compound (1.0 g.) and hydrogen peroxide—*tert*.-butanol (3M; 3 c.c.) was treated with a solution of osmium tetroxide in *tert*.-butanol (2%; 4 drops) and set aside at 0° for 48 hours. (A similar experiment carried out for the most part at room temperature was negative in result.) Removal of solvent under diminished pressure gave a syrup which, after attempts at solidification had been unsuccessful, was heated for 30 minutes in acetic anhydride with a trace of concentrated sulphuric acid. Working up in the usual way furnished a dark oil. The isolation of a pure product

was hampered by considerable tar formation concomitant with the reaction and subsequent distillation; and work on it was terminated while still incomplete.

cis-Hex-3-ene-1:6-diol

A solution of hex-3-yne-1:6-diol (1.5 g.) in ethyl acetate (60 c.c.) was shaken under hydrogen with palladium-charcoal (10%; 150 mg.) until 1 mol. of hydrogen had been absorbed. Removal of catalystaand solvent followed by distillation gave cis-hex-3-ene-1:6-diol (1.1 g.) as a viscous hygroscopic liquid. b.p. 86-87°/0.3 mm., n_D^{15} 1.4750 (Found: C, 61.1; H, 10.8. $C_6H_{12}O_2$ requires C, 62.0; H, 10.4%). The bisphenylurethane prepared at room temperature crystallised from light petroleum (b.p. 80-100°) in needles, m.p. 107-108° (Found: C, 68.0; H, 6.2; N, 8.2. $C_{20}H_{22}O_4N_2$ requires C, 67.8; H, 6.3; N, 7.9%).

trans-Hex-3-ene-1:6-diol

Hex-3-yne-1:6-diol (1.0 g.) was transferred from a dropping funnel to a stirred solution of sodium (1.0 g.) in liquid ammonia (50 c.c.) by dripping liquid ammonia through it. The cooled (alcohol—carbon dioxide) reaction mixture was stirred for a further two hours and was then decomposed by the addition of ammonium chloride (4 g.). After evaporation any residual ammonia was removed by warming under reduced pressure and the residue was

extracted with boiling ethyl acetate. Filtration, evaporation, and distillation gave trans-hex-3-ene-1:6-diol (0.8 g.) as a viscous, hygroscopic liquid, b.p. 88-90°/0.3 mm., n_D^{18} 1.4747 (Found: C, 60.7; H, 10.7%). The bisphenylurethane crystallised from toluene in plates, m.p. 161-162° (Found: C, 68.0; H, 6.1; N, 7.9%).

erythro-Hexane-1:3:4:6-tetrol

A cooled (0°) mixture of cis-hex-3-ene-1:6-diol (0.8 g.) and hydrogen peroxide—tert.-butanol (2.7M; 3 c.c.) was treated with a solution of osmium tetroxide in tert.-butanol (2%; 0.1 c.c.) and set aside at 0° for 24 hours. Removal of solvent under diminished pressure gave a viscous syrup which crystallised after several days at 0°. Crystallisation from ethanol gave erythro-hexane-1:3:4:6-tetrol (0.2 g.) as prisms, m.p. 113-114° (Found: C, 48.0; H, 9.2. $C_6H_{14}O_4$ requires C, 48.0; H, 9.4%). Treatment of the tetrol with hydrogen chloride and aqueous formaldehyde (40%) by the method of Wolfrom, Lew, and Goepp (23), namely by passing hydrogen chloride at 0° and thereafter heating the mixture to 85°, gave a brown oil which was isolated with ether; evaporation gave an oil that rapidly solidified. Crystallisation from ethanol gave the dimethylene compound in plates, m.p. 146-147° (Found: C, 55.4; H, 7.8.

$C_8H_{14}O_4$ requires C, 55.2; H, 8.1%). This derivative was obtained both from the pure crystalline tetrol and from the syrupy residue derived from the mother-liquors.

threo-Hexane-1:3:4:6-tetrol

(a) Treatment of trans-hex-3-ene-1:6-diol (0.1 g.) and hydrogen peroxide—tert.-butanol (2.7M; 0.4 c.c.) with a solution of osmium tetroxide in tert.-butanol (2%; 1 drop) as described for the cis-diol yielded a viscous syrup.

(b) An aqueous solution of hydrogen peroxide (2M; 50% excess) containing cis-hex-3-ene-1:6-diol (0.86 g.) and tungsten trioxide (0.2% w/v) was prepared by dissolving tungsten trioxide monohydrate (13 mg.; from sodium tungstate by acidification with concentrated hydrochloric acid) in hydrogen peroxide (30%; 1.3 c.c.), diluting with water, adding the cis-diol, and finally diluting with water to 5.7 c.c. (cf. Mugdan and Young, ref. 15). The mixture was heated at 65-70° for 2½ hours and was then evaporated to a clear viscous syrup.

An attempt was made to crystallise a dimethylene derivative obtained by treatment of the syrupy threo-tetrol with formaldehyde and hydrogen chloride according to the procedure already described, but the only substance resulting was a brown oil.

P A R T T W O

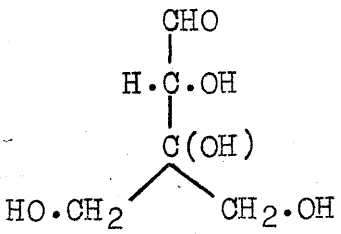
The Synthesis of Apiose and Cordycepose

PART TWO. The Synthesis of Apiose and Cordycepose

HISTORICAL INTRODUCTION

Only a few of the naturally occurring sugars so far identified have a branched carbon chain. Two of them, apiose (XXXII, p. 39) and hamamelose (XXXIII), both isolated from plants, have long been known and the other five, streptose (XXXIV), hydroxystreptose (XXXV), cordycepose (XXXVI), mycarose (XXXVII), and cladinose (XXXVIII) have been discovered in the last ten years as components of antibiotics elaborated by micro-organisms. All are aldoses with a chain of five, six, or seven carbon atoms: among them streptose and hydroxystreptose are peculiar in being aldehydo-sugars, since they have two C-formyl groups, one of which is not in a lactol ring. In addition, it may be noted that apiose and cordycepose both possess the isoprene skeleton. Dendroketose (XXXIX) is a wholly synthetic branched-chain hexose; another wholly synthetic product is 1:2:3:3-tetra-C-methylglycerose (XL). The remaining branched-chain sugars reported in the literature are not naturally occurring and have been prepared from other sugars. They are the 1:1-dialkyl-D-fructoses; 1:1-diphenyl-D-fructose and a few other phenyl sugars;

the 6:6-dialkyl-D-galactoses; and more recently a derivative of 3-ethyl-D-altrose. This last compound appeared in a complicated reaction of diethylmagnesium and



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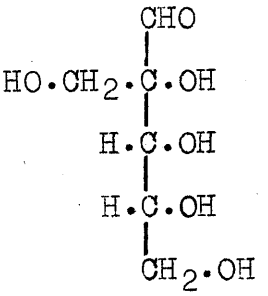

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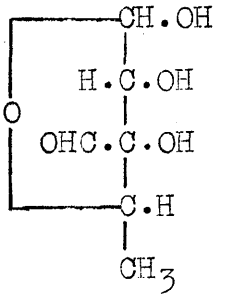

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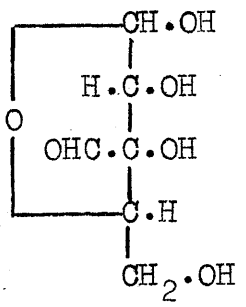
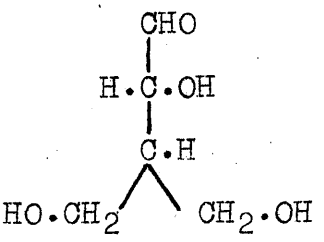
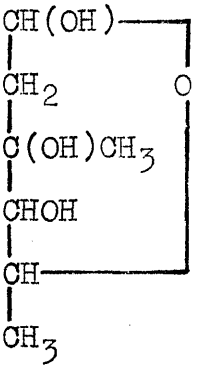


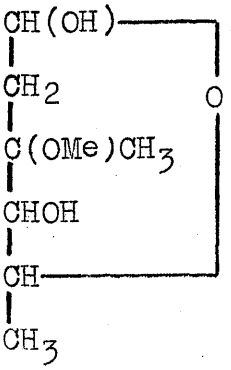
(XXXV)



(XXXVI)



(XXXVII)



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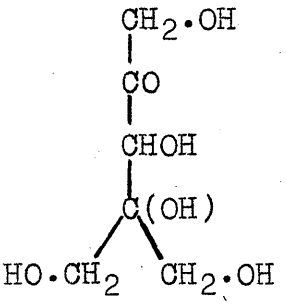
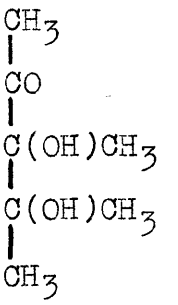
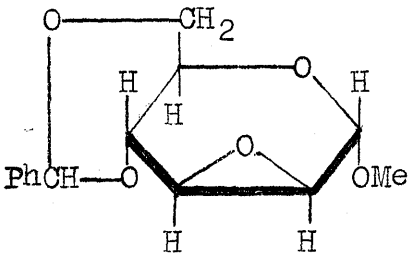


(XXXIX)

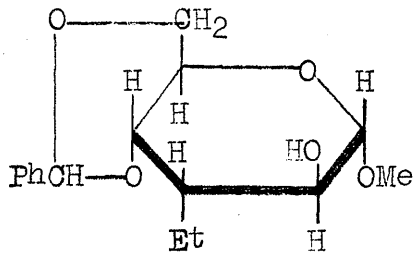


(XL)

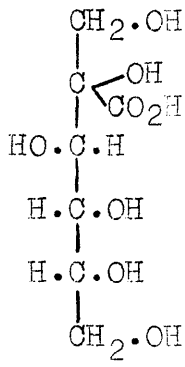
methyl 2:3-anhydro-4:6-O-benzylidene-α-D-mannoside (XLI). In ether the sole product was methyl 4:6-O-benzylidene-3-deoxy-3-C-ethyl-α-D-altroside (XLII)(30).



(XLI)



(XLII)



(XLIII)

The "d-fructoheptose" reported by Fischer (31) has not been fully investigated. "d-Fructoheptonic acid" or fructose carboxylic acid (XLIII) is obtained by the action of hydrogen cyanide and hydrogen chloride on D-fructose and is reducible to 2-methylhexanoic acid (32).

None of the natural branched-chain sugars has hitherto been synthesised, although attempts have been made to synthesise the leading member, apiose. Possible building units for the biosynthesis of apiose, hamamelose, and streptose have been suggested and discussed by Hough and Jones (33). In view of the current interest in the products of micro-organisms, and the additions to the series that have appeared as a result, it would seem that the neglect of the chemistry and biochemistry of branched-

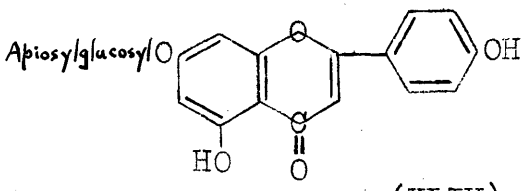
chain sugars must end..

The principal eight sugars mentioned will now be treated in more detail. The synthesis of the ketose (XL) will be discussed on p. 62.

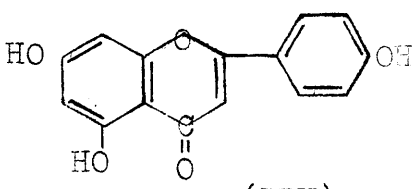
Apiose

The first of these compounds to be discovered was apiose (XXXII). This compound is obtained as a dextrorotatory syrup, together with D-glucose, on hydrolysis of the flavone glycosides extracted by alcohol from the parsley plant, Apium petroselinum (Umbelliferae). After early work (1836-1867) on parsley extracts, culminating in Lindenborn's obtaining a crystalline mixture of glycosides, crude apiin (34), Vongerichten's investigations (1876-1906) were responsible for the discovery of apiose and the elucidation of its structure. Vongerichten was hampered by working on mixtures of closely related glycosides, and by being at first unaware of this complication. In an article in 1949 reviewing the work of Vongerichten and his contemporaries and of Schmidt (a later contributor in this field), Hudson (35) usefully defines names occurring in the literature which varied in meaning throughout the course of these researches; and he also distinguishes between Vongerichten's proved results and his partly intuitive inferences, of which many were shown by later knowledge to be correct.

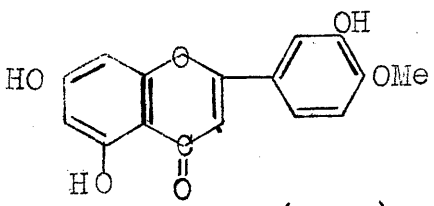
If Hudson's names for the substances are adopted, Vongerichten's work shows that the usually predominating parsley glycoside is apiin (XLIV), the aglycone being apigenin, 5:7:4'-trihydroxyflavone (XLV) (synthesised by Kostanecki and his co-workers, ref. 36, and shown by them to be the principal component of natural crude apigenin), and the carbohydrate fragment consisting of a disaccharide apiosylglucosyl radical attached to position 7 of apigenin. The other glycoside that Vongerichten eventually considered



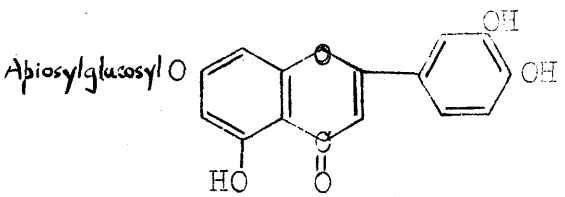
(XLIV)



(XLV)



(XLVI)



(XLVII)

was present in the mixture was named petroselinin by Hudson. Vongerichten assumed that it differed in structure from apiin only in the nature of the aglycone but there is no evidence for this assumption. The aglycone was isolated (37) and was proved to be a monomethyl ether of luteolin. Vongerichten's evidence indicated that it was probably 5:7:3'-trihydroxy-4'-methoxyflavone (XLVI) (later known as diosmetin), and this was established in 1930 by the

synthesis of this structure by Robinson and his co-workers (38).

The recent discovery of the physiological effects of certain flavone glycosides (39) has stimulated further work on the extracts of parsley. This is not wholly consistent with the earlier conclusions, a result which can possibly be ascribed to variations in the type and age of the plants used. There have been two reports of the hitherto unaccomplished isolation of apiin in a pure state. Gupta and Seshadri (40) purified apiin *via* its lead salt, but were unable to isolate a second glycoside and considered that apiin was the only component in extracts from certain parsley plants grown in Delhi. Nordström, Swain, and Hamblin (41), by paper chromatography of a crude apiin mixture, separated two main components in the approximate ratio of 5:1; these were shown to be respectively apiin [7-(apiosylglucosyl)apigenin], and 7-(apiosylglucosyl)luteolin (XLVII). These workers also reported the tentative identification of an apigenin glucoside and a naringenin derivative among the minor components (2—5% of the original crude mixture), but could find no diosmetin glycosides present in the seed, leaf, or stalk of the material examined.

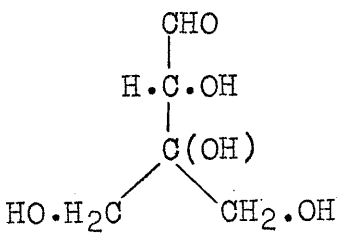
It was not until 1901 that Vongerichten discovered that the crystalline parsley glycosides contained another sugar unit in addition to D-glucose. Analytical figures

for crude apiin, interpreted on the basis that apiin alone was being analysed, had indeed indicated two hexose residues associated with apigenin. In that year Vongerichten (42) identified D-glucose by crystallisation and also showed that the other sugar was a pentose, which he named apiose. This assignment rested on the analysis of the crystalline phenylosazone and crystalline p-bromo-phenylosazone which he prepared from the reducing sugar obtained by partial hydrolysis of crude apiin. The pentose appeared to be of a new kind, since it did not yield furfural on acid treatment. He rejected his first idea (42) that it was a ketose and proved (43) that it was an aldopentose by oxidation with bromine water to an amorphous acid and consequent analysis of derivatives of this oxidation product.

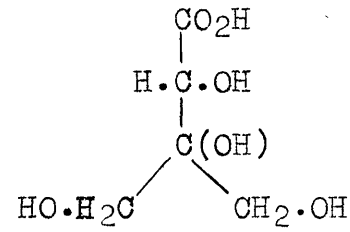
Investigation of the structure of apiose was made by Vongerichten (44) and twenty-four years later by Schmidt (45). Both used the same conversion, namely the reduction of calcium apionate to isovaleric acid, to show that apiose had the branched chain structure (XXXII), but Schmidt's method was the more rigorous. Whereas Vongerichten reduced amorphous calcium apionate prepared from amorphous apiose, Schmidt first purified apiose by regenerating it by formaldehyde treatment from its crystalline α-benzyl-α-phenylhydrazone (m.p. 137-138°)

(44), before oxidising it with barium hypoiodite to apionic acid (XLVIII). He succeeded in crystallising calcium apionate and on reducing this with hydriodic acid and phosphorus obtained a 4% yield of purified volatile acid, identified as isovaleric acid by means of its p-bromophenacyl ester (m.p. 68°).

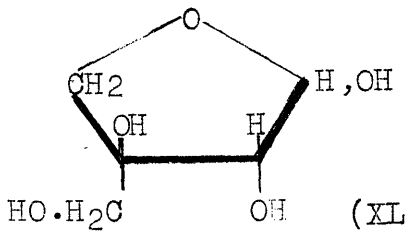
To establish the configuration of apiose, Schmidt applied Levene's empirical generalisations, the salt-acid rule and the phenylhydrazide and amide rules (46), to derivatives of apionic acid, which has only one asymmetric centre. Comparison of the rotations of the acid, the sodium salt, and the phenylhydrazide, with the rotations of the corresponding derivatives of other α-hydroxy carboxylic acids indicated that apionic acid has the D-configuration. The configuration of the aldehyde form of apiose is therefore considered to be represented by (XXXII). The apiofuranose ring may therefore be either (XLIX) or (L). (XLIX) is



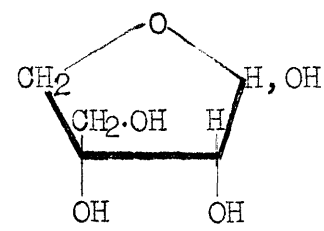
(XXXII)



(XLVIII)



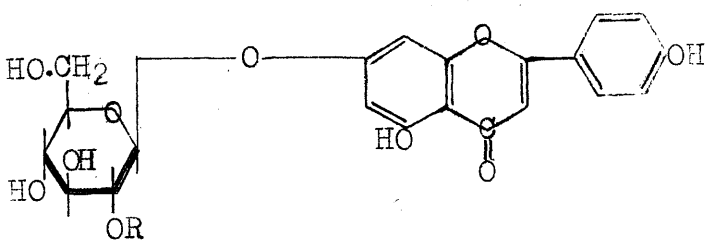
(XLIX)



(L)

α- or β-D-apio-L-furanose in the nomenclature recently suggested (47) for sugars which like apiose produce on cyclisation two new centres of asymmetry.

The structure of apiin is now known to be represented by (LI), where R = apiofuranosyl and the configuration of the glycosidic linkage between the apiose and the D-glucose residue has not yet been determined. Vongerichten's conclusion (42)(from experiments involving the partial methylation of crude apiin) that the D-glucose



(LI)

residue was joined to position 7 of apigenin has been confirmed both by Narasimhachari and Seshadri (48), who completely methylated apiin and obtained on hydrolysis 5:4'-di-O-methylapigenin, and by Nordström, Swain, and Hamblin (41), who obtained the same compound on hydrolysis of completely methylated D-glucosylapigenin. The observations of Vongerichten (42)(44)(and the later workers) on D-glucosylapigenin show that the anomeric linkage is β and that the D-glucose portion exists as a pyranose ring. The 1:2 linkage between the apiofuranosyl residue and a

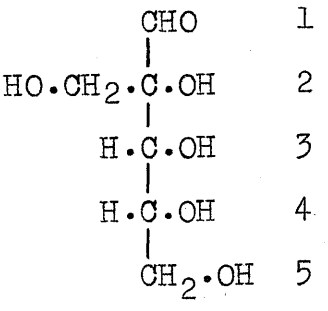
glucopyranosyl residue has been proved by Hemming and Ollis (49), who have isolated 3:4:6-tri-O-methyl-D-glucose and 5-4'-di-O-methylapigenin from the hydrolysis product of completely methylated crude apiin.

Since the completion of the present studies, a further source of D(+)-apiose has appeared, with its identification by Bell, Isherwood, and Hardwick (50) as the main monosaccharide liberated, on mild acid hydrolysis, from both leaves and naturally "retted" residual fibres ("marine fibre") of the monocotyledon, Posidonia australis (Potamogetonaceae). These authors describe two new crystalline compounds, the di-O-isopropylidene derivative of D-apiose, and the inactive apiose 2:5-dichlorophenylosazone.

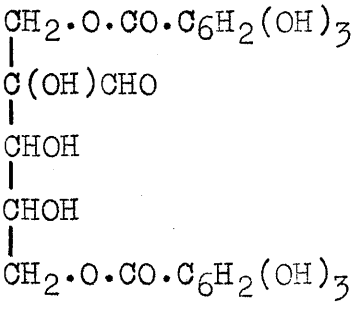
Hamamelose

A crystalline tannin, hamameli tannin (51), occurs in the bark of the shrub Hamamelis virginica L. (Witch Hazel), and has been shown to be di-O-galloyl-hamamelose (52). Hamamelose itself is a laevorotatory syrup which Freudenberg (53) obtained along with two molecules of gallic acid by the action of tannase on his improved preparations of hamameli tannin. His researches showed that the sugar was a hexose yielding no osazone and capable of being oxidised to a hexonic acid and thus led him

to favour the structure (XXXIII) for hamamelose. Schmidt's reduction of hamamelonic acid to 2-methylpentanoic acid (54) confirmed this, and further proof was furnished by the same worker's synthesis of hamamelonic acid from D-arabulose by the action of hydrogen cyanide on the latter (55). This synthesis established the configuration at C_3 and C_4 in hamamelose; previously Schmidt's comparison of the rotations of hamamelonic acid and derivatives with the rotations of fructose carboxylic acids and derivatives had indicated that hamamelose had the D-configuration at C_2 also (56). Therefore hamamelose was seen to be 2-C-hydroxymethyl-D-ribose (XXXIII). Freudenberg (57) considered that both primary hydroxyl groups of hamamelose are esterified in hamameli tannin, as in formula (LII).



(XXXIII)



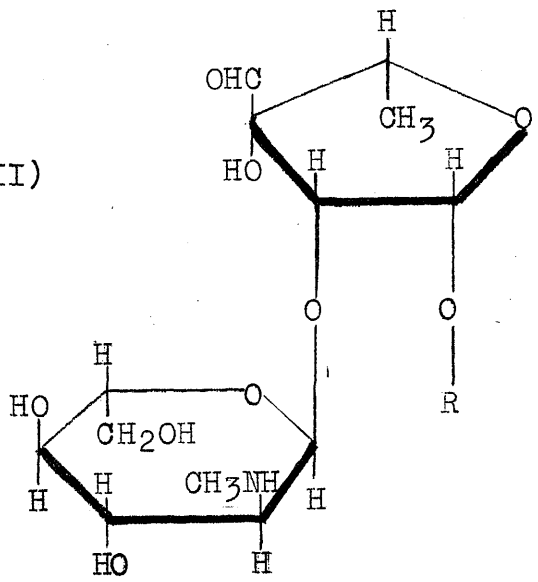
(LII)

Streptose

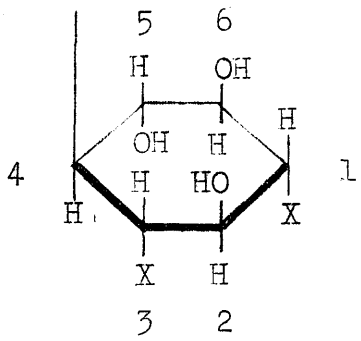
Streptomycin, one of the most studied antibiotics in recent years, was the first clinically important product of the actinomycetes to be discovered, and that group of

micro-organisms has since received much attention in the search for fresh antibiotic substances. Notable because of its low toxicity and selective activity against gram-negative bacteria, streptomycin was the subject of intensive chemical and biological investigation in the years immediately following its detection in the culture media of a soil organism, Streptomyces griseus, in 1944 (58). The outcome was the almost complete determination of the structure and configuration of this complex carbohydrate in an unusually short time.

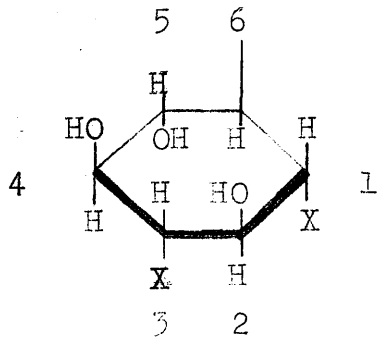
(LIII)



R =



or



X = —NH·C·NH$_2$ (with NH)

The structural formula (LIII, p. 49) of streptomycin shows that the central portion is a branched-chain sugar, L-streptose (XXXIV, p. 53). Because of its instability, streptose was not itself isolated, and that portion was the last structure to be established. The chief degradative procedures employed in elucidating the structure of streptomycin were acid hydrolysis, methanolysis, and mercaptolysis. By these means the weaker of the two glycosidic linkages was readily broken to yield streptidine (the strongly basic diguanidino-inositol portion) and the disaccharide streptobiosamine or derivatives thereof. Acid hydrolysis of methyl streptobiosaminide dimethyl acetal led to the decomposition of the streptose fragment of the disaccharide and the isolation after acetylation of the penta-acetyl derivative of a methylaminohexose, which was later shown to be N-methyl-L-glucosamine (59).

Thereafter identification of crystalline reduction and oxidation products comprised the method of defining the constitution of streptose. Hydrolysis following hydrogenolysis of the acetylated diethyl mercaptal of ethylthiostreptobiosaminide hydrochloride (LIV) gave N-methyl-L-glucosamine and crystalline dideoxydihydrostreptose (LV). The latter had two C-methyl groups and two hydroxyl groups. Oxidation by

periodic acid (one mol.) followed by hydrolysis gave glycollic aldehyde and acetylmethylcarbinol, showing that the hydroxyl groups were on adjacent carbon atoms; their cis-configuration was indicated by the formation of a complex with boric acid. The structure of streptosonic acid was shown by oxidation of its monolactone (obtained from tetra-acetylstreptobiosamine by oxidation and then hydrolysis) and its diamide. The monolactone consumed two mols. of periodic acid to give glyoxilic acid and oxalic acid; the diamide consumed two mols. to give acetaldehyde and no volatile acid.

Streptose could then be assigned the formula (LVI), based on the structures of dideoxydihydrostreptose (60)(61) and streptosonic acid (62).

The further stereochemical evidence required for establishment of the formula (XXXIV) comprised the degradation of streptose to 4-deoxy-L-erythrose to show the L-configuration about C_4 (63) and the application of Hudson's rules of rotation to the dextrorotatory hydrazide of dihydrostreptosonic acid to show the D-configuration about C_2 (64). Confirmation of the latter point was obtained with the degradation of N-acetyltetrahydrostreptobiosamine to L-glyceric acid (65).

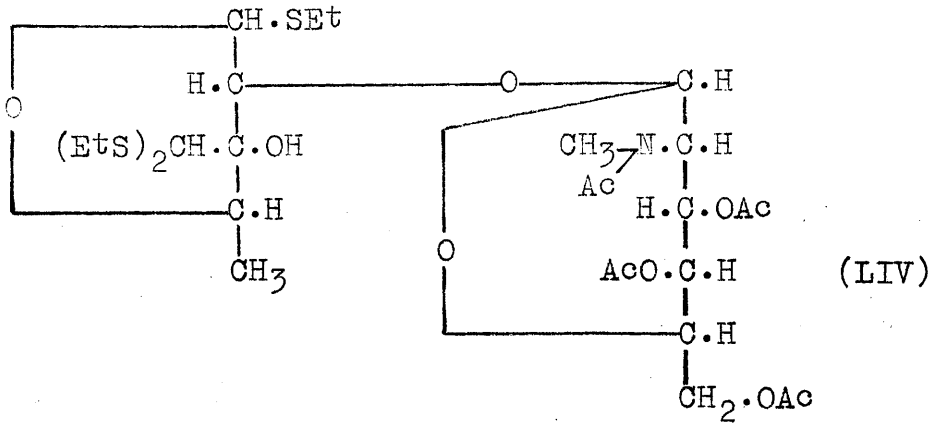
The linkage of N-methyl-L-glucosamine at C_2 of streptose was deduced from the demonstration (60)(61) that

the compound (LIV) after desulphurisation contained a free tertiary hydroxyl group, and the linkage of streptidine at C_1 of streptose was proved by degradation of dihydrostreptomycin (61)(66). Early calculations showed that both glycosidic linkages in streptomycin had the α-configuration (67), but these calculations depended on the assumption that the point of attachment of the streptobiosamine fragment to streptidine was at C_5 of the latter, i.e. the symmetrical point. Since later work showed that the fragment is unsymmetrically attached, i.e. at C_4 or C_6 (which are not sterically equivalent), Wolfrom and his co-workers have revised their calculations and have concluded (68) that in all probability the streptidine——streptose link is β-L and that in all probability the hexosamine——streptose link is α-L.

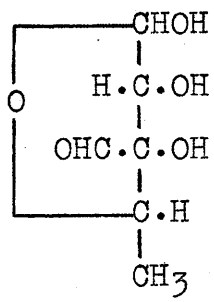
An interesting rearrangement of the branched-chain sugar to an unbranched-chain derivative occurs when a compound containing the streptose fragment with the formyl group at C_1 glycosidically combined and with the other formyl group free, e.g. streptomycin itself, is treated with aqueous alkali under relatively mild conditions; maltol (LVII) is thereby obtained (62)(69).

Several detailed reviews of the chemistry of streptomycin are available (70).

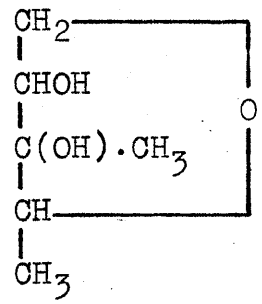
Closely related compounds are streptothricin,



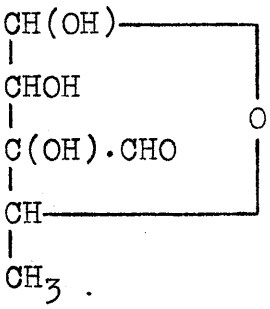
(LIV)



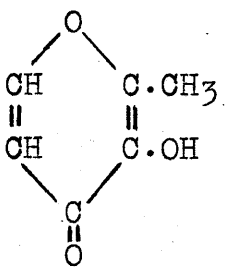
(XXXIV)



(LV)



(LVI)

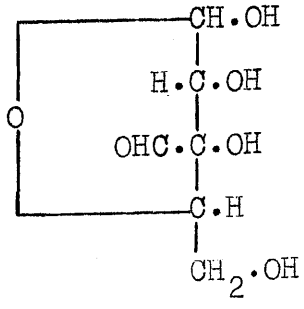


(LVII)

isolated earlier than streptomycin, from Streptomyces lavendulae, but neglected because of its excessive toxicity, mannosidostreptomycin or streptomycin B, in which D-mannose is linked glycosidically to the N-methylglucosamine portion, and dihydrostreptomycin, similar in effects to the parent compound and derived from it by catalytic hydrogenation of the aldehydo-group.

Hydroxystreptose

An antibiotic hydroxystreptomycin, distinguished from streptomycin by paper chromatography, is produced by an actinomycete, Streptomyces griseocarneus (found in Japanese soil) (71), and also by another strain of Streptomyces found in Chicago soil (72). The molecule differs from streptomycin in having an extra oxygen atom, the branched-chain sugar component being hydroxystreptose (XXXV).

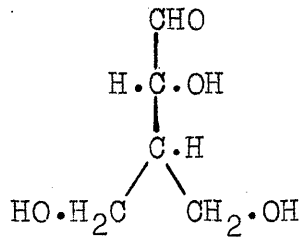


(XXXV)

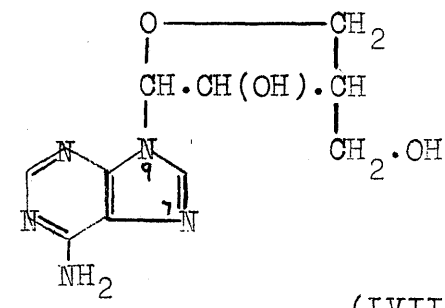
Cordycepose

In 1951 Cunningham, Hutchinson, Manson, and Spring (73) isolated from cultures of the mould Cordyceps militaris

(Linn.) Link. a crystalline antibiotic metabolic product which was named cordycepin. Degradative work by Bentley, Cunningham, and Spring (74) showed that cordycepin was a glycoside, 9-N-cordyceposyladenine (LVIII), yielding on acid hydrolysis adenine and a deoxypentose, 3-deoxy-D-apiose (XXXVI), which was given the name cordycepose.



(XXXVI)



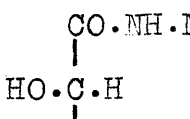
(LVIII)

The point of attachment of the carbohydrate group to the aglycone was limited to the 7- or 9- position by the deamination of cordycepin with nitrous acid to a product from which hypoxanthine was obtained by acid hydrolysis; the choice between these two was readily made by comparison of the ultra-violet absorption characteristics of cordycepin with those of appropriate adenine derivatives.

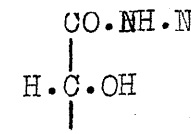
The analytical figures of the nitrophenylosazones (giving the characteristic blue colour with sodium hydroxide in aqueous ethanol) which formed slowly in hydrochloric acid from solutions of acid-hydrolysed cordycepin and either p-nitrophenylhydrazine or 2:4-dinitrophenylhydrazine

required that the parent sugar be a deoxypentose. The syrupy sugar was readily oxidised by bromine water to a lactone, characterised as a crystalline phenylhydrazide. Analysis of this compound showed it to be the derivative of a five-carbon acid; cordycepose was thus demonstrated to be an aldodeoxypentose. The osazone formation indicated that it could not be a 2-deoxy-sugar and the resistance of cordycepin itself to periodate oxidation left an aldo-3-deoxypentose as the sole remaining possibility.

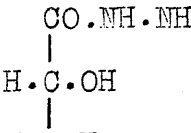
There are four possible straight-chain 3-deoxyaldopentonic acids, viz. D- and L-threo- and D- and L-erythro-$\alpha\gamma\delta$-trihydroxyvaleric acids. The corresponding phenylhydrazides (LIX) - (LXII) have been described by Nef (75).



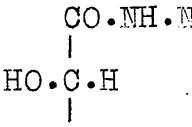
(LIX)



(LX)



(LXI)



(LXII)

Cordyceponic acid phenylhydrazide (m.p. 151°, $[\alpha]_D$ +26°) differed considerably in m.p. from D- and L-threo-$\alpha\gamma\delta$-trihydroxyvaleric acid phenylhydrazides (m.p. 110°; LIX and LX)

(75), and was shown to be different from L-erythro-αγδ-trihydroxyvaleric acid phenylhydrazide (m.p. 149°, $[\alpha]_D$ +4.5°; LXII)(75)(76) by a comparison with an authentic specimen of the latter. Since cordyceponic acid phenylhydrazide was not identical with any of the phenylhydrazides (LIX) to (LXII), the branched-chain structure (XXXVI) was assigned to the aldehyde form of cordycepose; cordycepose is thus (-)-3-deoxyapiose. As cordyceponic acid phenylhydrazide, in which there is only one asymmetric centre, was found to be dextrorotatory, the D-configuration as written in (XXXVI) is indicated by the phenylhydrazide rule. Cordycepin itself was formulated as (LVIII) where the configuration of the 1' and 3' centres is still unknown.

Mycarose

In 1953 there were reported (77) the isolation and structure of a crystalline sugar given the name mycarose (XXXVII). This sugar occurs as a fragment of the antibiotic carbomycin (78)(79) or Magnamycin*, a product of strains of Streptomyces halstedii. Magnamycin B, another antibiotic from Streptomyces halstedii, also yields mycarose (80).

A neutral oil and a crystalline base were obtained on acid methanolysis of Magnamycin: the former was shown

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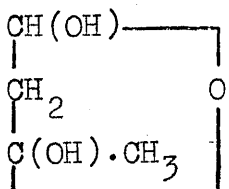
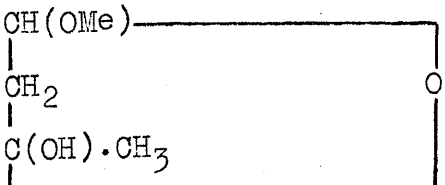
* Registered trade name of Chas. Pfizer & Co.

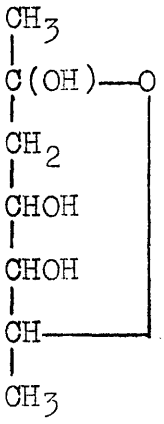
to be methyl 4-O-isovalerylmycaroside (LXIII) by the following degradative and analytical data. Alkaline hydrolysis of (LXIII) gave isovaleric acid and a mixture of anomeric methyl mycarosides (separable by fractionation into a crystalline isomer and a liquid isomer), from which the sugar itself, m.p. 128-129°, was obtained by acid hydrolysis. Mycarose, a heptose having two C-methyl groups, three active hydrogen atoms (Zerewitinoff) and a very slow reaction with hot Fehling's solution, consumed two mols. of periodate, with a yield of one mol. each of acetaldehyde and formic acid; acetoacetaldehyde (characterised by the formation of a 2:4-dinitrophenylmethylpyrazole) was produced when only one mol. of periodate was used for oxidation.



(XXXVII)



(LXIII)



(LXIV)

This evidence is satisfied by the formulations (XXXVII) and (LXIV); (LXIV) can be eliminated as oxidation

of mycarose by hypobromite readily yielded the lactone of a heptonic acid. The point of attachment of the isovaleryl group in (LXIII) is determined by the failure of this glycoside to react with periodate.

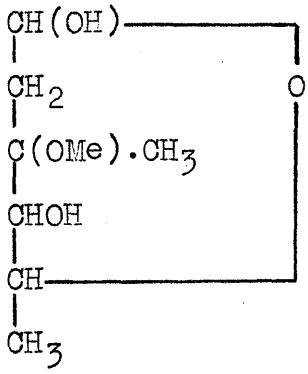
The configuration of mycarose is currently the subject of investigation by the same authors (77).

Cladinose

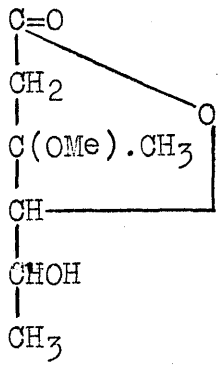
Erythromycin is an antibiotic produced by Streptomyces erythreus. One of the structural components isolated on mild acid hydrolysis of erythromycin is a sugar, cladinose (81)(82). Wiley and his co-workers (82)(83) have proved that the structure of this sugar is 3-O-methylmycarose (XXXVIII); its configuration has not yet been determined.

Cladinose was shown to contain two C-methyl groups and one methoxyl groups; the presence of two hydroxyl groups was shown by the formation of a diacetyl derivative and by the infra-red spectrum, which also indicated a hemiacetal structure. There was present a CH_3CHO— grouping with this oxygen in the hemiacetal ring since cladinose, but not methyl cladinoside with only one hydroxyl group, gave a positive iodoform reaction. Infra-red absorption showed further that the product of oxidation by bromine was mainly a γ-lactone. Ultra-violet absorption attributable to αβ-unsaturation appeared on base treatment of the lactone

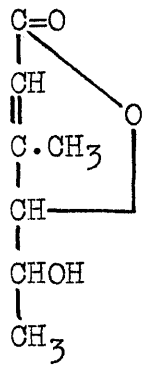
followed by neutralisation. Finally periodate oxidation of the unsaturated lactone gave acetaldehyde and β-formylcrotonic acid (LXV). A six-carbon chain in cladinose was thereby established, and the formation of the former showed that there was a C-hydroxyl group adjacent to the $CH_3.CHO$— fragment mentioned above. The formation



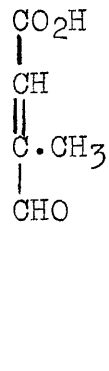
(XXXVIII)



(LXVI)



(LXVII)



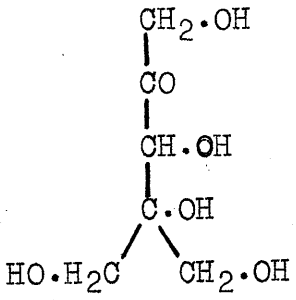
(LXV)

of β-formylcrotonic acid confirmed that αβ-elimination of methoxyl had occurred and also showed the C-methyl group in cladinose to be on position 3. (XXXVIII) is therefore the only possible structure for cladinose and the saturated and unsaturated lactones obtained in the degradation experiments are formulated (LXVI) and (LXVII).

Dendroketose

In the course of synthetic experiments, Utkin (84) isolated a ketose structurally related to apiose from the

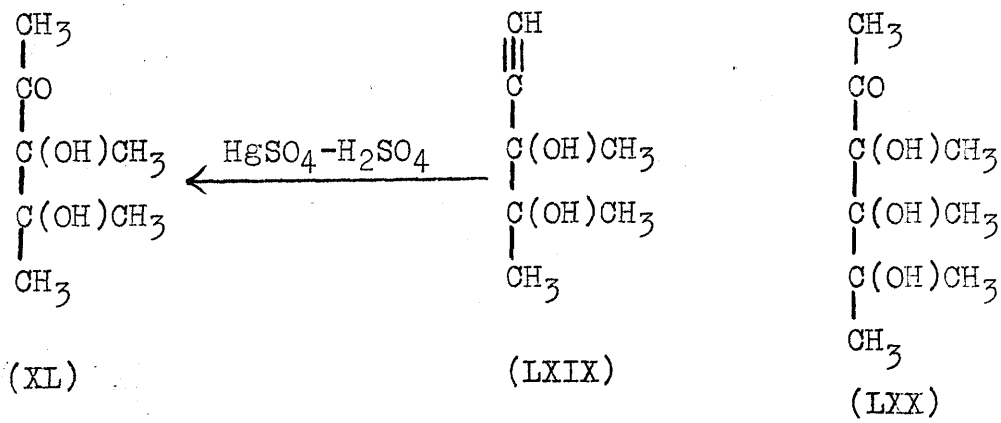
condensation in sodium hydroxide solution (0.05 N) of two molecules of sym.-dihydroxyacetone. He provisionally named the sugar dendroketose (XXXIX).



(XXXIX)

THEORETICAL

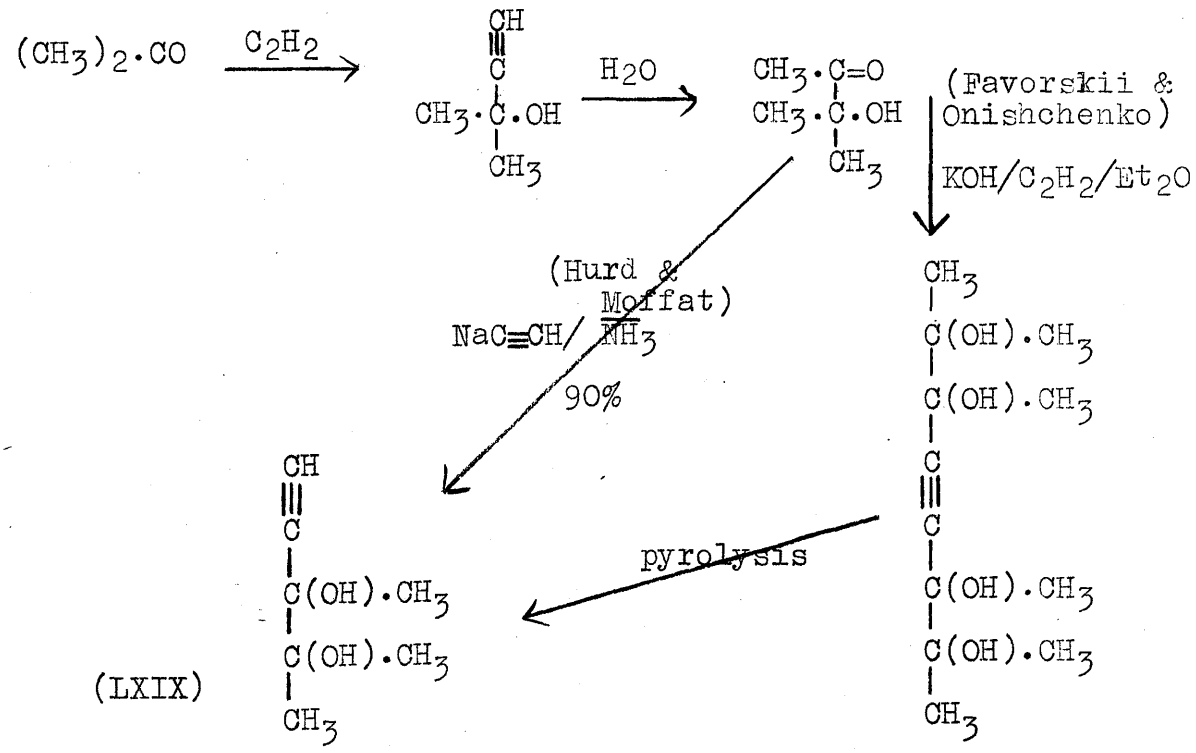
The previous success in applying acetylenic derivatives to the synthesis of straight-chain carbohydrates suggested the extension of these methods to the branched-chain carbohydrates. The only previously reported application of such intermediates to the synthesis of branched-chain sugars was the preparation of 3:4-dihydroxy-3:4-dimethylpentan-2-one (XL) by hydration of 2:3-dimethylpent-4-yne-2:3-diol (LXIX). Thus a



(XL) (LXIX) (LXX)

general procedure was established for the synthesis of a certain type of poly-C-methylketose, employing successively two reactions of triply-bonded compounds, (i) the condensation of a carbonyl compound with acetylene and (ii) the hydration of the resulting ethynylcarbinol to an α-hydroxyketone. Hurd and Moffat (85) set out to synthesise a monosaccharide whose alcohol groups were

tertiary, i.e. the dihydroxyketone (XL), which may be regarded as 1:2:3:3-tetra-C-methylglycerose, and to that end prepared the acetylenic glycol (LXIX). Shortly after their work had been stopped at this point by the outbreak of war, the complete synthesis was published by Favorskii and Onishchenko (86). The different reactions used by the two groups to obtain (LXIX) are shown below.



Hurd and Moffat pointed out the possible extension of the synthesis, namely that starting from (XL) a similar sequence of steps should produce a penta-C-methyltetrose (LXX, p. 62).

This type of synthesis has been examined recently by Hickinbottom, Hyatt, and Sparke (87) in the course of a

study of branched-chain hydrocarbons. They have reported some limitations of the synthesis of α-hydroxyketones as follows, R or R' (or both) being a tertiary or secondary alkyl group:

$$RR'CO \longrightarrow RR'C(OH).C{\equiv}CH \longrightarrow RR'C(OH).CO.CH_3$$

Concurrently with the present work, an attempt to synthesise apiose has been in progress (88) according to the following scheme, commencing with a modified Reformatsky reaction.

$$(CH_2.OBz)_2.CO + Zn + CH_2Br.C{\equiv}CH \longrightarrow (CH_2.OBz)_2.C(OH).CH_2.C{\equiv}CH$$

$$\downarrow -H_2O$$

(LXXI) $(CH_2.OBz)_2.C{=}CH.C{\equiv}CH$

(LXXII) $(CH_2.OBz)_2.C(OH).C(OH).C{\equiv}CH$

$$\downarrow \begin{array}{l}1.\ \text{Benzoylation}\\ 2.\ \text{Semihydrogenation}\\ 3.\ \text{Hydroxylation}\end{array}$$

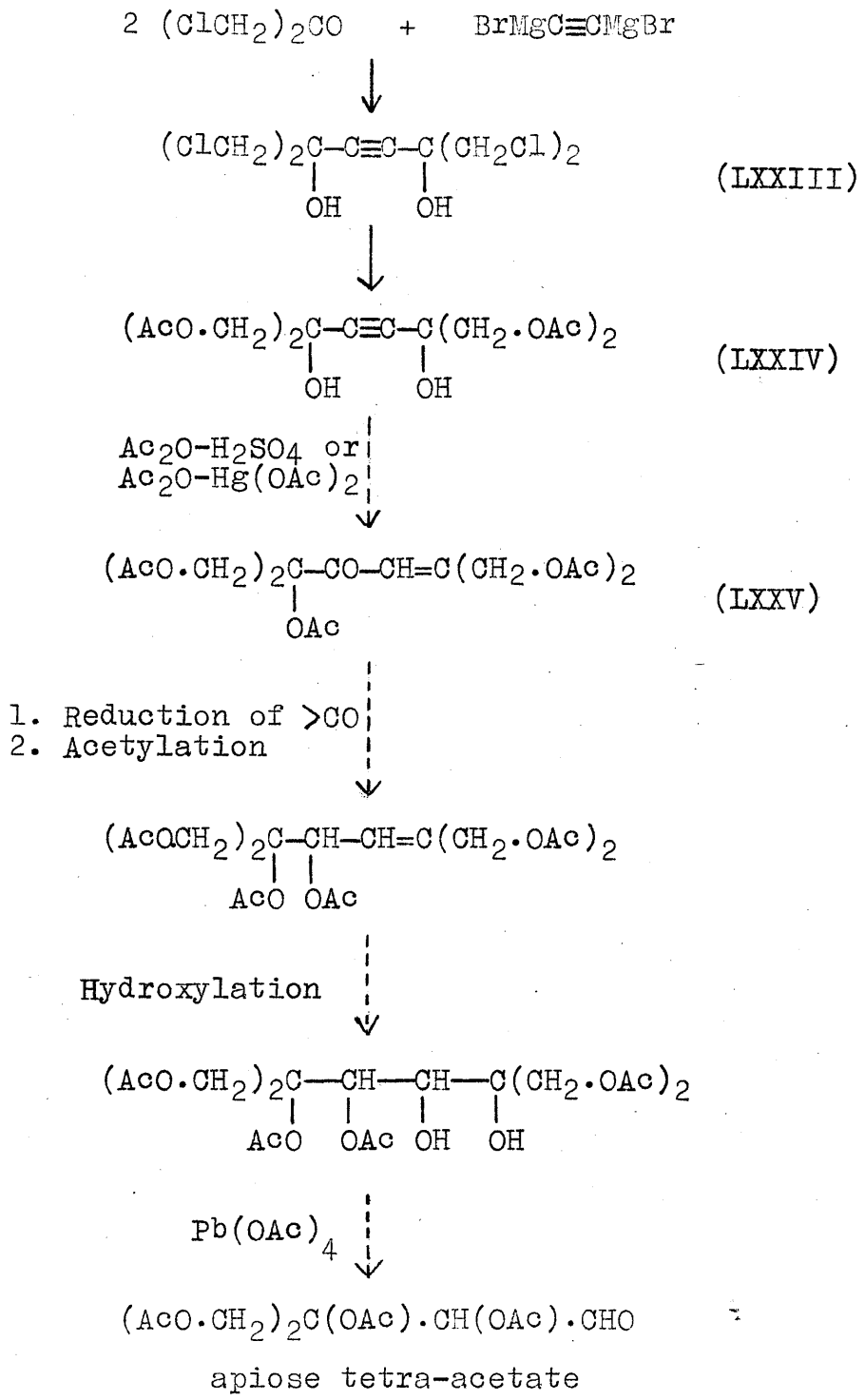
$$(CH_2OBz)_2.C(OBz).CH(OBz).CH(OH).CH_2.OH$$

$$\downarrow \begin{array}{l}1.\ HIO_4\\ 2.\ \text{Hydrolysis}\end{array}$$

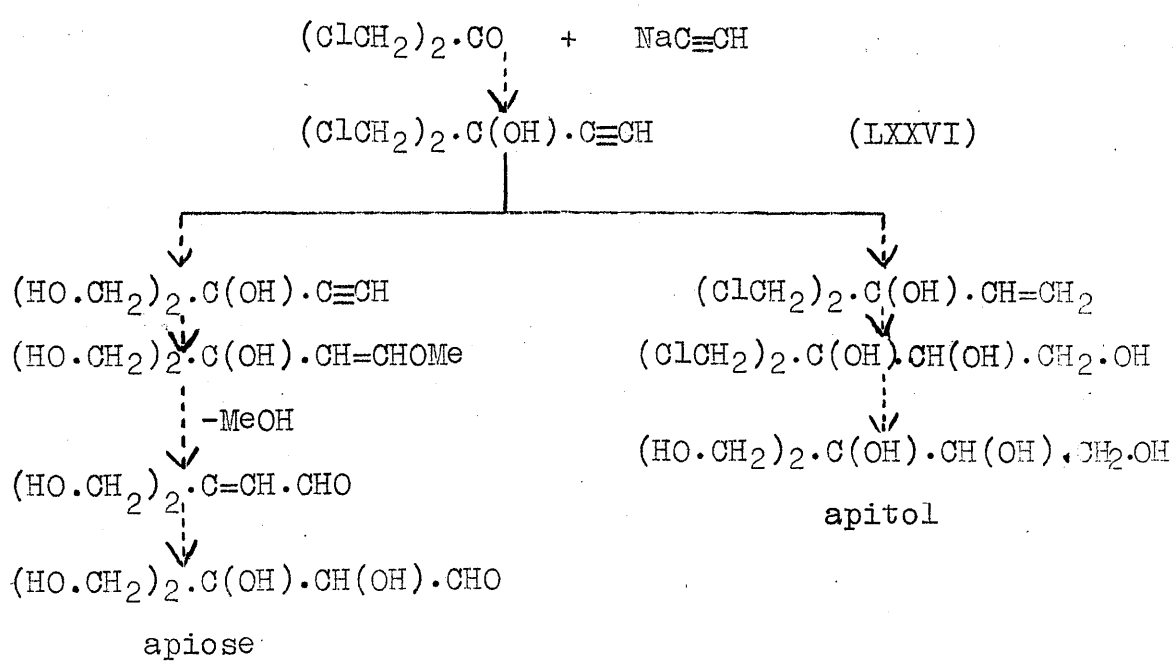
$$(CH_2.OH)_2.C(OH).CH(OH).CHO$$

The oxidation of the vinylacetylene (LXXI) to give the glycol (LXXII) is very difficult because of the bulk of the neighbouring benzoyloxy groups. It is understood that (LXXII) has been obtained, in crystalline form albeit in very small yield, by the use of osmium tetroxide.

The first contemplated synthesis of apiose, outlined on the flow sheet on p. 66, was clearly rather long, but quite justifiable if the steps following the initial condensation were found to give good yields of conveniently characterisable compounds. The reaction of acetylenedimagnesium bromide with sym.-dichloroacetone produced the expected acetylenic glycol (LXXIII), obtained eventually as pure, low-melting crystals. The extensive formation of by-products, including the corresponding ethynyl carbinol and probably dichloroacetone pinacol, lowered the yield (to 39%) and purification of the desired product was tedious and involved considerable loss of material. Replacement of chlorine by the action of potassium acetate or the milder reagent silver acetate gave the tetra-acetate (LXXIV), which could not be crystallised. The hexacetate similarly was an oil. The anionotropic rearrangement—acetylation reaction (LXXIV) $\longrightarrow$ (LXXV) did not proceed smoothly with acetic anhydride - sulphuric acid; acetic anhydride - mercuric acetate was tried (cf. 89) but gave a very low yield of inhomogeneous product. In view of these difficulties in the early stages, this synthesis was abandoned in favour of a more promising approach.

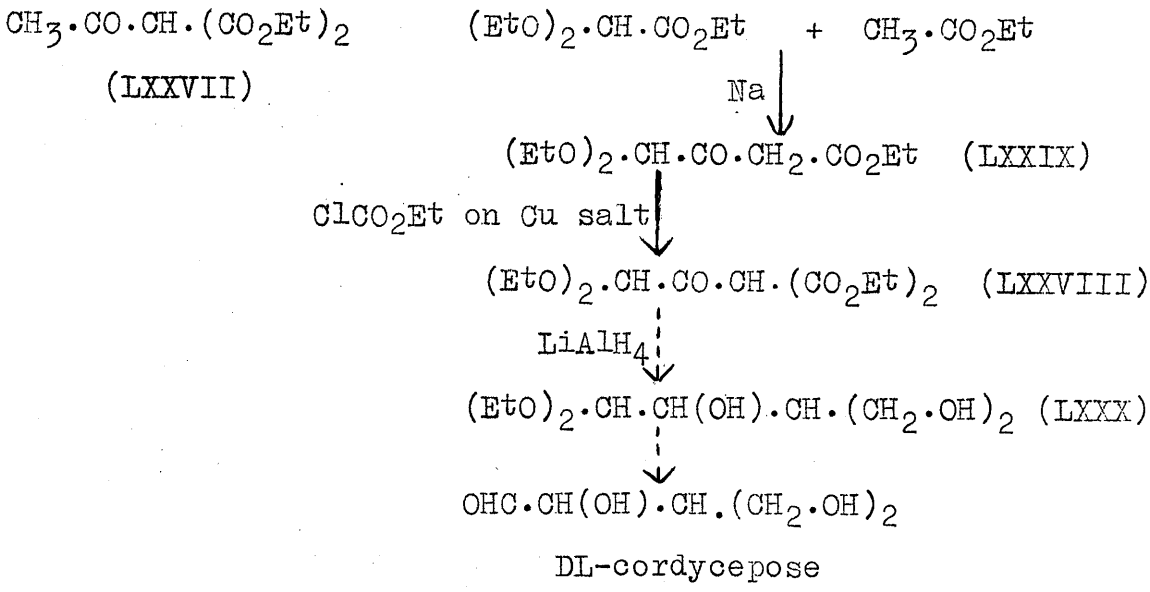

2 (ClCH2)2CO + BrMgC≡CMgBr
(ClCH2)2C—C≡C—C(CH2Cl)2
OH OH
(LXXIII)
(AcO.CH2)2C—C≡C—C(CH2.OAc)2
OH OH
(LXXIV)
Ac2O-H2SO4 or
Ac2O-Hg(OAc)2
(AcO.CH2)2C—CO—CH=C(CH2.OAc)2
OAc
(LXXV)
1. Reduction of >CO
2. Acetylation
(AcOCH2)2C—CH—CH=C(CH2.OAc)2
AcO OAc
Hydroxylation
(AcO.CH2)2C—CH—CH—C(CH2.OAc)2
AcO OAc OH OH
Pb(OAc)4
(AcO.CH2)2C(OAc).CH(OAc).CHO
apiose tetra-acetate

The reaction of sodium acetylide with sym.-dichloroacetone in liquid ammonia was next examined, since the carbinol (LXXVI) was thought to be a suitable precursor of apiose, and also of the sugar alcohol apitol. The synthetic routes envisaged from (LXXVI) were:



It was found however that the reaction in liquid ammonia did not produce the carbinol (LXXVI): the activity of the chlorine atoms was sufficient to cause reaction with the solvent. The very recent discovery of the preparation and use of acetylene monomagnesium bromide (90) would obviously lead to a very convenient preparation of the carbinol (LXXVI).

Some investigation was made of the following non-acetylenic approach to cordycepose:

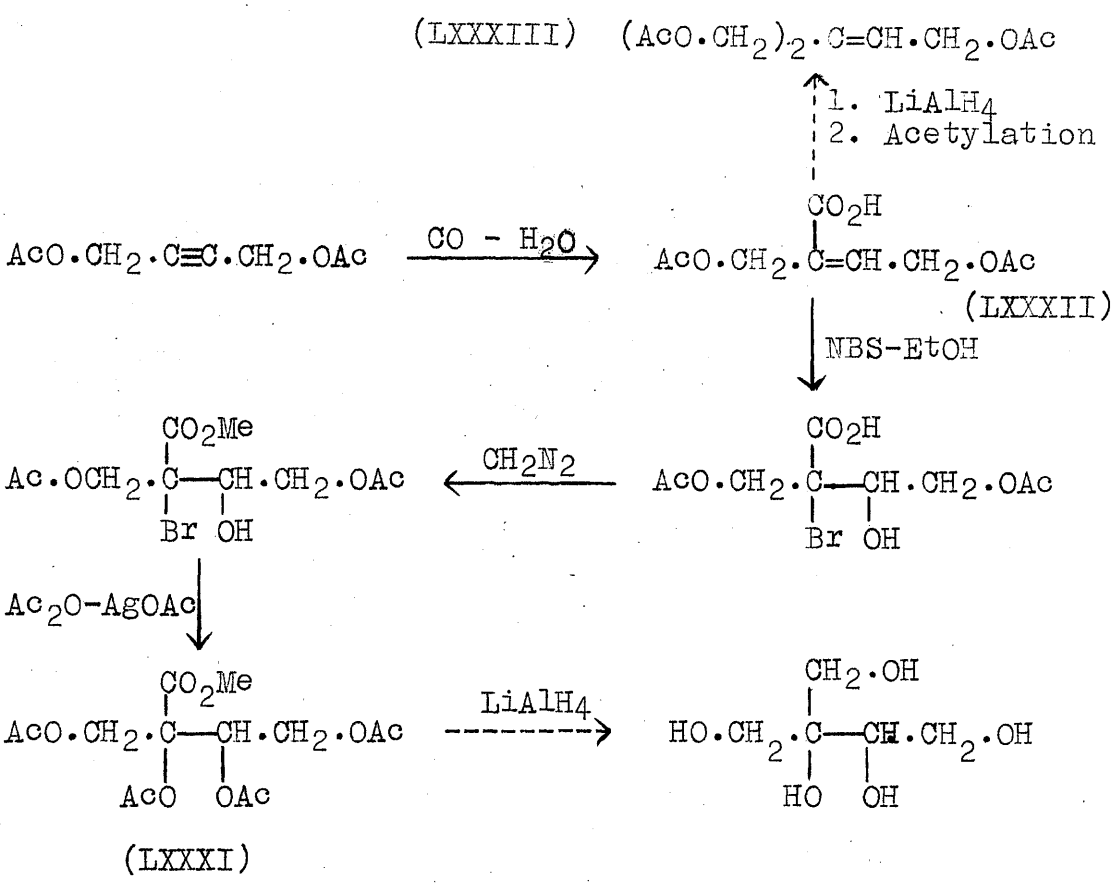


Diethyl acetomalonate (LXXVII) was prepared by Nef (91) by the reaction of ethyl chloroformate and the copper salt of ethyl acetoacetate. The aim here was to fabricate the cordycepose skeleton by preparing similarly diethyl γ:γ-diethoxyacetomalonate (LXXVIII) from ethyl γ:γ-diethoxy-acetoacetate (LXXIX). Reduction of (LXXVIII) by lithium aluminium hydride should then furnish DL-cordycepose diethyl acetal (LXXX). The condensation of ethyl acetate and ethyl γ:γ-diethoxyacetate to give (LXXIX) has been described by Dakin and Dudley (92). The copper salt of (LXXIX) was formed and its reaction with ethyl chloroformate examined. The maximum yield possible in this reaction is 50% as the product, being a stronger acid,

forms a copper salt at the expense of the unreacted diethoxyacetoacetic ester. In practice the yield was very much smaller, and its improvement beyond 15% and the isolation of a pure sample of the product were not achieved before work on this line was halted temporarily pending the availability of a further supply of the starting material. This compound, ethyl diethoxyacetate, is obtained by a laborious preparation from dichloroacetic acid. As it happened, however, the success of the synthesis of cordycepose from bromoacetal (to be described later) was assured before the resumption of work took place.

The synthesis of the alcohol corresponding to apiose, pentahydroxy<u>iso</u>pentane, or apitol, was considered worthy of attention. Although it was not thought likely that a conversion from apitol into apiose could be achieved, the apitol would have been, if crystalline, a useful derivative for the characterisation of the syrupy apiose once synthesised. Consequently two routes were explored, but neither was promising enough to warrant exhaustive study.

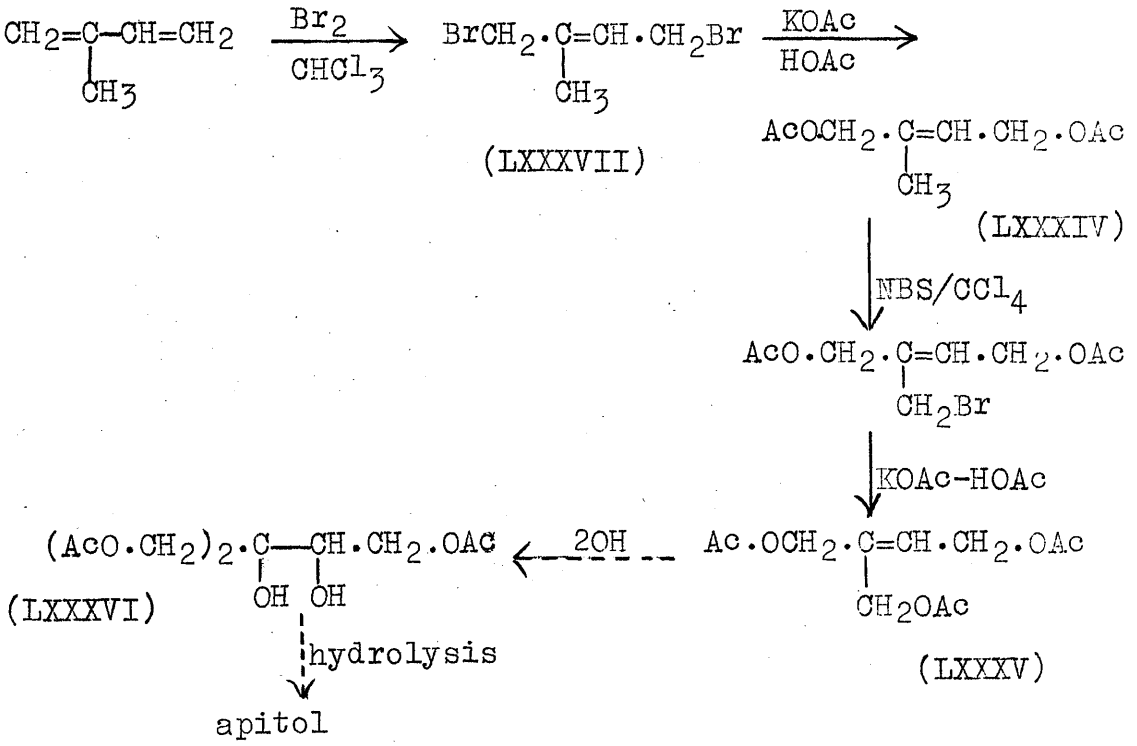
The first route, tested on a small scale as far as the tetra-acetate (LXXXI, p. 70), was the following, starting from the readily available but-2-yne-1:4-diol:



By the standard carbonylation reaction, but-2-yne-1:4-diol diacetate and nickel carbonyl yield the ethylenic acid (LXXXII)(93). Reduction of (LXXXII) with lithium aluminium hydride did not proceed smoothly. [The expected acetylated product of such a reduction (LXXXIII) would have given apitol triacetate on hydroxylation.] Addition of hypobromous acid (17) by means of N-bromosuccinimide in water was followed immediately by esterification of the hydroxy-acid. Acetylation with silver acetate—acetic anhydride was expected to give the tetra-acetate (LXXXI). Had this compound been characterised, its reduction with lithium aluminium hydride

would have been carried out to obtain apitol in one stage. The products however were all syrupy and there was evidence of decomposition and rearrangement, which hindered the isolation of pure compounds.

The other approach to apitol which was examined began from isoprene. Shepard and Johnson (94) prepared



1:4-diacetoxy-2-methylbut-2-ene (LXXXIV) by the 1:4-addition of bromine to isoprene followed by the replacement of bromine by treatment with potassium acetate. It was proposed to brominate (LXXXIV) with N-bromosuccinimide, replace the halogen with acetoxyl, and then hydroxylate the resulting triacetate (LXXXV) to obtain apitol triacetate

(LXXXVI). The preparation of the diacetate (LXXXIV) was accordingly repeated and unsuccessful attempts were made to obtain a pure sample of the ethylenic triacetate (LXXXV).

Shepard and Johnson (loc. cit.) reported the purification of the dibromide (LXXXVII) to be tedious and wasteful, because of decomposition during distillations, and preferred to convert the crude dibromide directly into the diacetate. This finding was confirmed both in the case of the dibromide and in the case of the tribromide. Shepard and Johnson also reported that repeated treatment of the dibromide with potassium acetate——acetic acid did not remove the last traces of bromine. It was found in the present work that these traces were not removable from the product by careful fractional distillation, but more serious was the difficulty encountered in attempting to debrominate completely the reaction product at the next stage. Not only was there present some substance containing unreactive bromine, but also the reactions and distillations were accompanied by the formation of appreciable quantities of decomposition products. It is probable that the reaction of two molecules of the dibromide to give one molecule each of isoprene and the 1:2:3:4-tetrabromide was responsible for the difficulty in obtaining a bromine-free product at the first stage;

the reason for the greater difficulty at the second remains uncertain. Numerous variations in reaction conditions and reagent proportions were made in attempts to decrease the formation of by-products, and some improvement was achieved before it was decided that further experiments in connection with this proposed synthesis were not justified.

The Synthesis of Apiose and Cordycepose from Bromoacetal and Ethyl Malonate

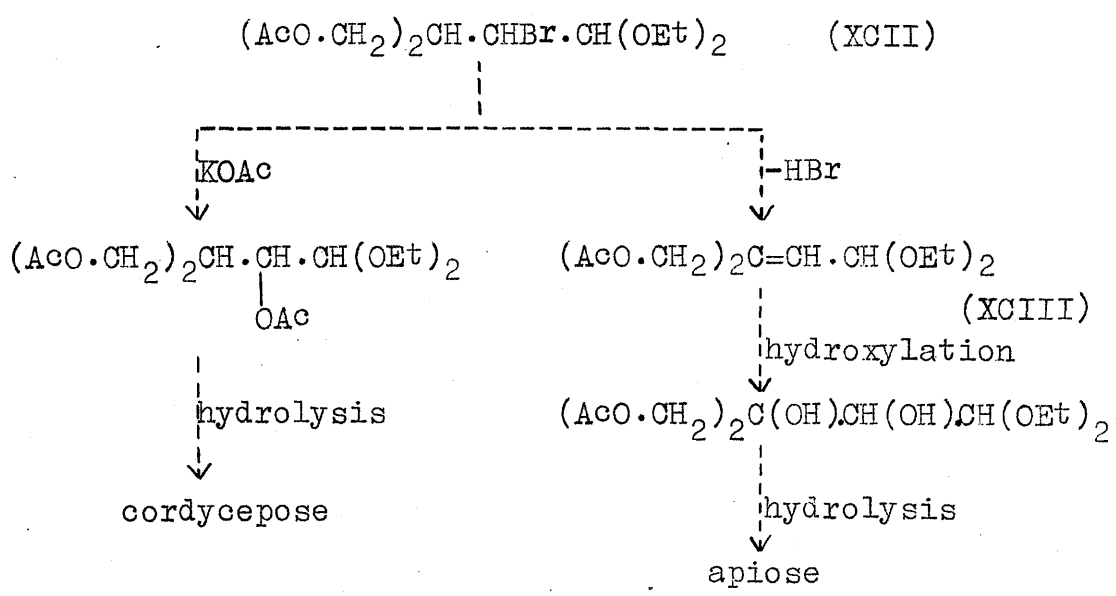
The unsuccessful experiences already detailed with acetylenic precursors led to the reluctant abandoning of this line of approach and the adoption of more conventional techniques. The stages of the synthesis of cordycepose and of the synthesis of apiose, both of which are described in this section, are depicted on the flow sheets on pages 86 and 87. The routes finally developed to give successful syntheses were not wholly the ones envisaged at the outset, and the variations, along with the results which prompted or forced them, will be discussed as they arise.

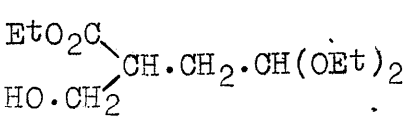
The condensation of the readily-available (95) bromoacetal with ethyl sodiomalonate has been carried out by Perkin and Pink (96) and more recently by Bowman and

Fordham (97); the higher-yielding earlier method employing simple pressure equipment was adopted. Smooth reduction of the resulting diesteracetal (LXXXVIII) with ethereal lithium aluminium hydride followed, and the product, 1:1-diethoxy-3:3-di(hydroxymethyl)propane (LXXXIX) was carefully separated by distillation from a small quantity of by-product, presumably 1:1-diethoxy-3-ethoxycarbonyl--3-hydroxymethylpropane (XC, p. 75). It was found that if more dilute ethereal solutions of diesteracetal and hydride were mixed, the amount of this semi-reduced compound was increased. A similar reduction of the homologous diesteracetal to give 1:1-diethoxy-4:4-di-(hydroxymethyl)butane has been done, but in 33% yield only (98). Attempts were made to prepare crystalline derivatives of the diol (LXXXIX); in every case the reaction was unsuccessful or the compound could not be solidified. By treatment with acetic anhydride in pyridine the diol was converted into the diacetate (XCI), from which a low-melting 2:4-dinitrophenylhydrazone (m.p. 56-57°), the derivative of the corresponding aldehyde 3:3-di(acetoxymethyl)propanal, was obtained by treatment with an acetic acid solution of 2:4-dinitrophenylhydrazine.

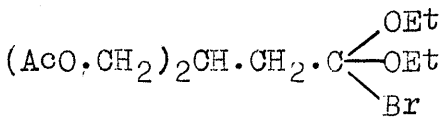
Direct α-bromination of (XCI) was next envisaged, to produce the key compound, the bromo-acetal

(XCII, p.75). It can readily be seen that replacement of the bromine atom in this compound would furnish a derivative of cordycepose, whilst dehydrobromination would produce an unsaturated acetal (XCIII) hydroxylation of which would give an apiose derivative.





(XC)



(XCIV)

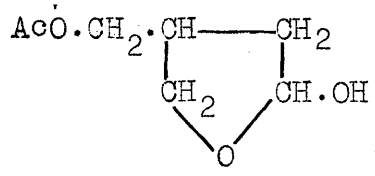
The most promising procedure for bromination of (XCI) appeared to be the one described by Simpson (99), who brominated 1:1:3-triethoxypropanal in good yield, although he could not isolate the pure bromo-compound because of decomposition on distillation. Similar results were obtained eith the acetal in the present case, but the very

unreactive bromine atom in (XCII) could not be successfully replaced. Reaction conditions could not be devised such that a large enough proportion of the bromine reacted with potassium acetate, without causing side reactions and decomposition. It is suspected that the crude bromination product (XCII) contained a small amount of a substance having labile bromine, probably (XCIV, p. 75) (100). This would account for the ready initial debromination to a small extent which occurred. Bromination by the action of N-bromosuccinimide on the acetal (100) was likewise unsuccessful for the production of a pure compound.

Introduction of a double bond into the skeleton (XCI) already constructed was considered to provide, in addition to a precursor of cordycepose, a means of indirectly preparing a halogen-substituted compound useful for the approach to apiose. Enol acetates have been derived from aldehydes and ketones by treatment with acetic anhydride catalysed by potassium acetate or p-toluenesulphonic acid (101), and by treatment with isopropenyl acetate (102). Hydrolysis of the acetal (XCI) to the corresponding aldehyde (XCV, p. 77) was attempted for this purpose. Hurd and Saunders(103) hydrolysed sensitive acetals by treatment with a cold

aqueous solution of tartaric acid. Here reaction proceeded to a sufficient extent at 20° — but not at 0°. The product was not the expected diacetoxyaldehyde (XCV) but a monoacetyl compound which is considered to possess the structure (XCVI). The yield after purification

$(AcO.CH_2)_2CH.CH_2.CHO$ (XCV)



(XCVI)

was rather low and, as this method was soon abandoned because of the success of the methods later described, no attempt was made to improve the preparation.

The acetic anhydride—potassium acetate procedure was applied to the acetal (XCI) itself without effecting any appreciable conversion. Acetic anhydride—p-toluene-sulphonic acid on the other hand was rather too vigorous and led to much resinification although the vinyl ethyl ether, 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene (XCVII), was obtained in small yield. Doubtless some improvement could be expected with p-toluenesulphonic acid under milder conditions, but it was felt better to seek a less

$(AcO.CH_2)_2CH.CH=CH.OEt$ (XCVII)

$(AcO.CH_2)_2CH.CH=CH.OAc$ (XCVIII)

drastic catalyst, and to this end hydrated ferric chloride

was added to the mixture of the acetal and acetic anhydride at 50°. 28% of the acetal was recovered, but no other product was recognisable. Since the vinyl ethyl ether (XCVII) and the enol acetate (XCVIII, p. 77) were apparently equally satisfactory for the purposes required, attention was turned to the preparation of the former.

The conversion of acetals into vinyl ethers has been described by Claisen (104) who used acetyl chloride——pyridine, by Flaig (105) who used silica gel, active alumina, or phosphate-silicate catalysts, and by other groups (106)(107) using sodium hydrogen sulphate. The acetyl chloride——pyridine method failed with the acetal (XCI) but successful results were obtained with sodium hydrogen sulphate. At first the technique of Voronkow (107) was followed, the acetal being heated with a catalytic quantity of sodium hydrogen sulphate in a good still with a low still-head temperature, but it was soon found that the reaction went very rapidly to completion at 100° and that the determining factor for a high yield of the vinyl ethyl ether (XCVII) was the time taken for the removal of liquid from the catalyst. By using a large enough flask to contain the froth accompanying the evolution of ethanol, and a short, wide still-head, and completing the heating and distillation of 15 g. in nine

minutes, yields as high as 83% could be obtained. Prolonged heating with sodium hydrogen sulphate was avoided, to prevent elimination of one molecule of acetic acid as well as the molecule of ethanol, with formation of a diene and polymerisation. (XCVII) yielded the same 2:4-dinitrophenylhydrazone (from aqueous acetic acid solution) as the acetal (XCI) did in the same conditions, thus furnishing proof that the pyrolysis had caused no skeletal rearrangement.

Oxidation of the vinyl ethyl ether (XCVII) with perbenzoic acid gave the epoxy-ether (XCIX, p. 86), which was hydrolysed with aqueous alcoholic hydrochloric acid to yield a solution of (±)-cordycepose. The sugar was isolated as a colourless syrup and characterised as its p-nitrophenylosazone; natural (-)-cordycepose p-nitrophenylosazone was prepared from a sample of cordycepin kindly provided by Dr. H.R. Bentley. Since osazone formation removes the asymmetry of the apiose and cordycepose molecules, the natural D-sugar and the synthetic (±)- sugar may be compared by means of such derivatives. The identity of the p-nitrophenylosazones was shown by their mixed m. p. and the single-band chromatogram obtained from a mixture of the two.

(±)-Cordycepose was derived from the vinyl ethyl

ether (XCVII) by a second oxidation method, iodine silver benzoate in benzene being used to form the dibenzoate (C, p. 86) which was hydrolysed with hydrochloric acid. The p-nitrophenylosazone obtained from this product was identical with the specimens already mentioned.

An unsuccessful attempt was made to prepare cordycepose p-bromophenylosazone. The directions of Bentley, Cunningham, and Spring (74) were followed, but the principal (and the only isolated) product of this reaction mixture of cordycepose, p-bromophenylhydrazine, sodium acetate, and aqueous acetic acid was N-acetyl-N'-p-bromophenylhydrazine. This acetyl derivative, cream-coloured except when freshly purified, agreed in m. p. and appearance with the osazone described by the above authors, and the calculated analytical figures for the two compounds are sufficiently close to permit confusion. There are other pentoses quoted in the literature as forming a p-bromophenylosazone having a similar m. p. (ca. 160°) and lacking strong colour; it is possible that this is not the only instance where confusion has occurred.

The vinyl ethyl ether (XCVII) was used in the synthesis of apiose also. The first approach thence to be studied began with bromination of (XCVII) by N-bromo-succinimide, to give the tertiary bromide (CI). It was

intended either to replace the bromine atom in (CI) by an acetoxy-group to obtain the ethylenic compound (CII) (or CIII), or, in the probable event of anionotropic rearrangement occurring in the case of the ether such as would be expected in the case of the corresponding enol acetate, to treat with ethanol to obtain the $\alpha\beta$-unsaturated acetal (XCIII).

$(AcO.CH_2)_2C{=}CH.CH(OEt)_2$ (XCIII)

$(AcO.CH_2)_2C(Br).CH{=}CH.OEt$ (CI)

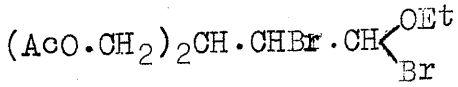
$(AcOCH_2)_2C(OAc).CH{=}CH.OEt$ (CII)

$(AcO.CH_2)_2C{=}CH.CH\langle^{OEt}_{OAc}$ (CIII)

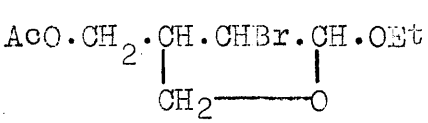
It is seen that (CII), (CIII), or (XCIII) would yield on hydroxylation a compound from which apiose could be obtained on hydrolysis. Neither of these bromination-debromination processes, however, appeared to lead to a single product. Hydrogen bromide was lost if distillation of the product of bromination was attempted; reaction of this crude substance with ethanol containing calcium carbonate to take up the displaced hydrogen bromide was tried both at room temperature and the boiling point with less than 20% conversion, and distillation of the product was again accompanied by evolution of hydrogen bromide. In consequence neither the unsaturated acetal nor any other

compound was isolated. Replacement of the bromine by an acetoxy group was unexpectedly difficult, excessively prolonged treatment with silver acetate—acetic anhydride being required for complete debromination. Distillation then gave an unidentified, halogen-free, water-soluble, unsaturated product. It was concluded that the complications inherent in this reaction sequence rendered it unsuitable for the current synthetic purposes.

The potential value of the bromoacetal (XCII) as a precursor of apiose has been already stated, and the preparation of this compound from (XCVII) by addition of the elements of ethyl hypobromite was next investigated. One method studied was the direct addition of bromine to the double bond followed by reaction with ethanol. The preparation of crude dibromides of vinyl alkyl ethers by the action of bromine in ether at a low temperature has been described (108). The dibromide (CIV) thus prepared



(CIV)



(CV)

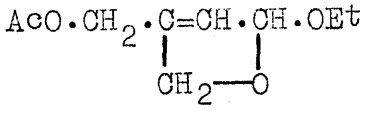
was treated, without previous isolation, with sodium ethoxide in ethanol, but hydrolysis of the ester groups resulted and, to prevent this, a suspension of sodium hydrogen carbonate was substituted for sodium ethoxide as

neutralising agent for the hydrogen bromide produced. A small yield of the desired acetal (XCII) was then obtained, but the main product was the cyclic acetal (CV, p. 82).

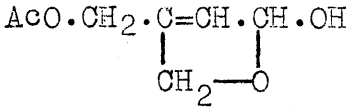
The acyclic bromo-compound was preferred, and was ultimately obtained from the vinyl ethyl ether (XCVII) by the action of N-bromosuccinimide in ethanol at 0°, a method in which the elements of ethyl hypobromite are added in a single step (105). Exclusion of moisture and control of temperature and acidity were necessary to prevent undesirable side reactions, chiefly oxidation of the ethanol by N-bromosuccinimide, hydrolysis by the acid formed in consequence of such oxidation, and elimination of ethyl acetate from the product (XCII).

Dehydrobromination of (XCII) by lithamide in liquid ammonia gave the αβ-unsaturated acetal (XCIII, p. 87) Various reagents for this dehydrobromination were examined since it was particularly important for separation of the desired product that completion of the reaction be secured and formation of by-products be minimised, in view of the close physical constants of the liquids concerned. The mildest technique, vacuum distillation from calcium carbonate, was ineffective, and heating with collidine, pyridine, or anhydrous lead acetate in pyridine for moderate periods abstracted approximately 50% only of the bromine present. Heating under nitrogen with potassium

tert.-butoxide (109) in tert.-butanol, benzene, or pyridine, with or without subsequent re-acetylation with acetic anhydride—pyridine, was much more successful in dehydrobrominating but inferior to the low-temperature lithamide—liquid ammonia method for the provision of a single product. With the latter reagent, the time of contact was varied to obtain the maximum removal of bromine uncomplicated by other reaction. Even then the compound (XCIII) proved to be very labile when distilled, ethyl acetate being evolved with the formation of cyclic products such as (CVI) and (CVII).



(CVI)



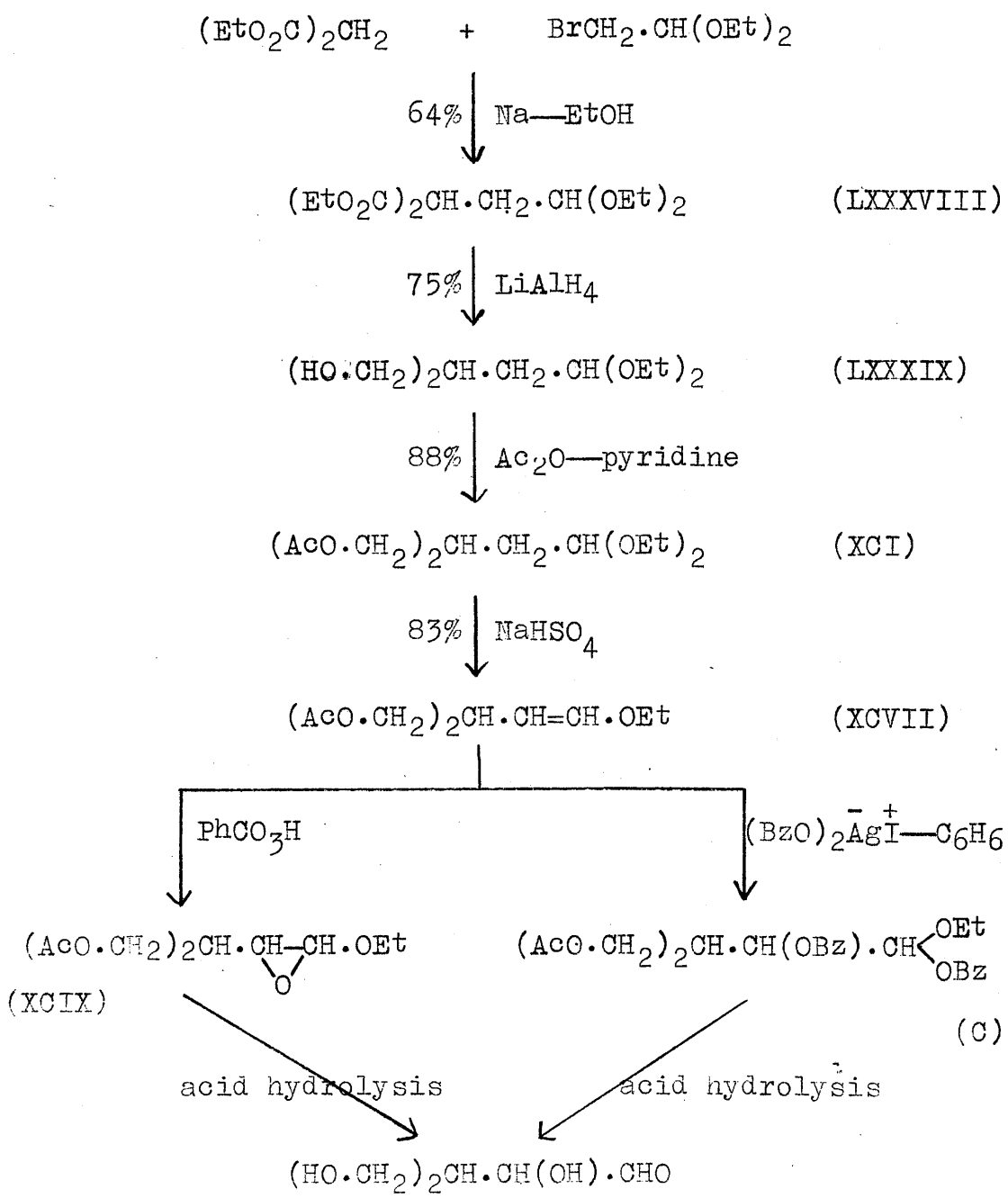
(CVII)

Because of the hindered nature of the double bond of the acetal (XCIII), neutral potassium permanganate (13)(12) was used in preference to other oxidising agents for hydroxylation. The aqueous solution was buffered by passage of carbon dioxide (11). Acid hydrolysis of the product yielded crude (±)-apiose, characterised as its p-bromophenylosazone. This derivative and a specimen of the p-bromophenylosazone derived from natural (+)-apiose were shown to be identical by comparison of their X-ray powder photographs and a mixed melting point determination.

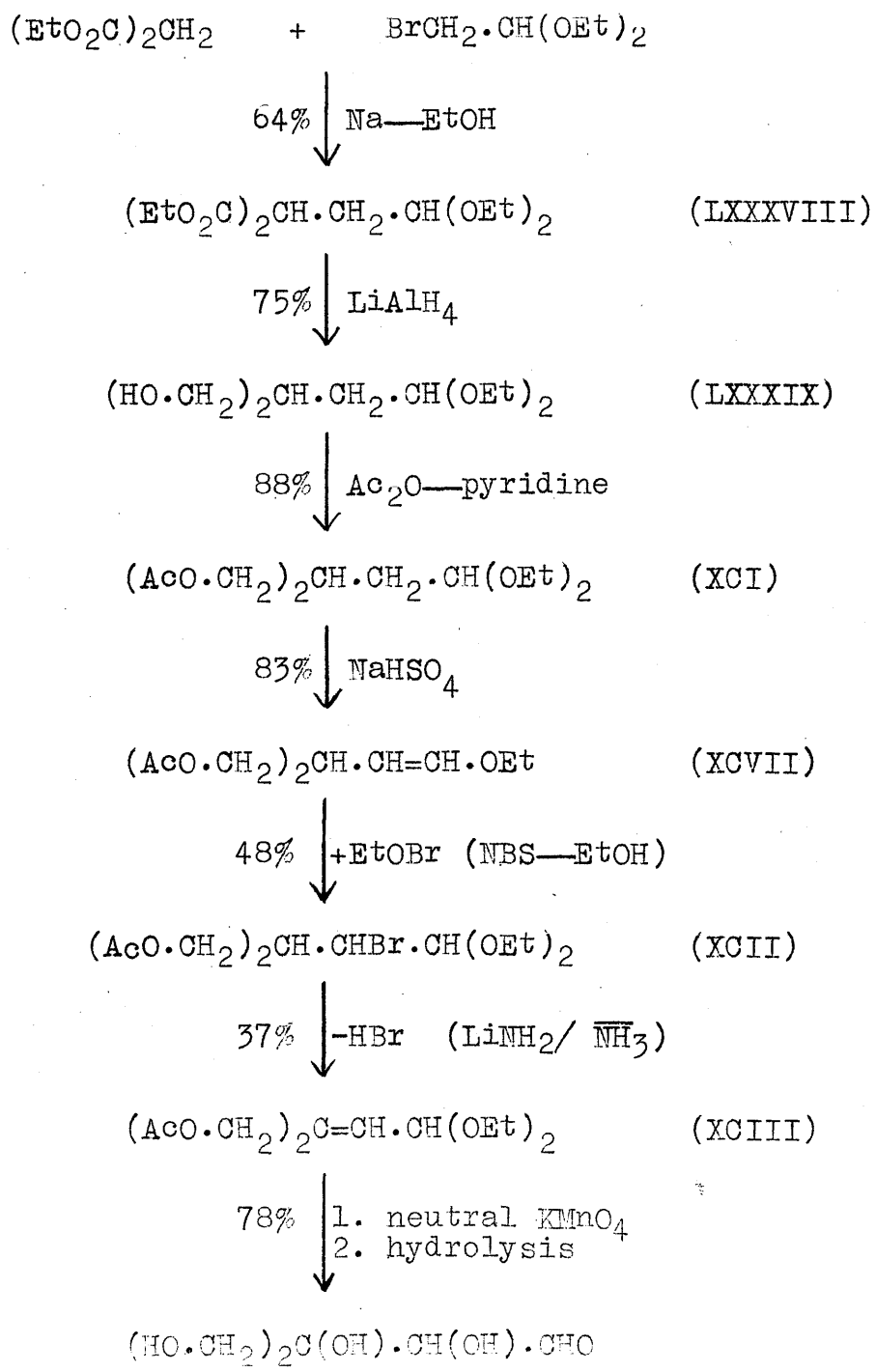
Thanks are expressed to Professor Wilson Baker, F.R.S., for the sample of the naturally-derived osazone, and to Professor J.M. Robertson, F.R.S., for arranging the X-ray determinations.

Confirmation of the structures assigned to apiose and cordycepose has thus been provided by synthesis. The overall yields of the racemic forms of apiose and cordycepose from bromoacetal were 5% and 20% respectively.

Synthesis of Cordycepose



Synthesis of Apiose



EXPERIMENTAL

1:1:4:4-Tetrachloromethylbut-2-yne-1:4-diol

A solution of ethylmagnesium bromide (from magnesium, 12 g.) in ether (200 c.c.) was prepared in the usual way. By the addition of hot dry benzene (400 c.c.) to the solution on the water-bath, followed by distillation of ether, most of the ether was replaced by benzene in order that the acetylenedimagnesium bromide to be produced should separate in a solid rather than in a gummy form. Pure dry acetylene was passed into the solution in benzene, with stirring, for 24 hours, and then sym.-dichloroacetone (63.5 g.) in benzene was added during 1 hour with vigorous stirring which was continued for a further 2½ hours. After this time the complex was decomposed with ice-cold dilute sulphuric acid added with stirring and cooling. The benzene layer was separated, and the aqueous layer was extracted several times with ether. The combined ether-benzene solution was washed with water and with sodium hydrogen carbonate solution, dried (Na_2SO_4), and evaporated, yielding a greenish-black oil (66 g.). The carbinol by-product and any unreacted ketone were removed by steam distillation. From the distillate by isolation with ether and distillation there was obtained a liquid (4.5 g.), b.p. 93-95°/20 mm., which was presumably

di(chloromethyl)ethynylcarbinol. The oil (54 g.) which was non-volatile in steam was isolated with ether and fractionally distilled to separate two products. The lower-boiling substance (16.4 g.) on redistillation was obtained as a viscous liquid, b.p. 92-93°/5 x 10^{-4} mm., n_D^{18} 1.5279 (Found: C, 28.8; H, 3.3. $C_6H_{10}O_2Cl_4$ requires C, 28.2; H, 3.9%), and was probably dichloroacetone pinacol. The higher-boiling compound (27 g., 39%), a viscous yellow liquid, b.p. ca. 140°/5 x 10^{-4} mm., crystallised on storage for several days at 0°. Recrystallisation from carbon tetrachloride gave 1:1:4:4-tetrachloromethylbut-2-yne-1:4-diol as prisms, m.p. 43-43.5° (Found: C, 34.6; H, 3.4. $C_8H_{10}O_2Cl_4$ requires C, 34.3; H, 3.6%).

Reactions of 1:1:4:4-tetrachloromethylbut-2-yne-1:4-diol

(a) Conversion into the hexacetate.——A mixture of the tetrachloroglycol (1 g.), potassium acetate (4.5 g.; fused), potassium iodide (0.2 g.), and acetic anhydride (20 c.c.) was boiled under reflux for 16 hours and then poured into water. The product was extracted with ether, and the extract was washed with water, sodium hydrogen carbonate solution, and finally water, and dried ($MgSO_4$). Evaporation of the solvent gave an oil (0.5 g.) which could not be crystallised even after distillation in a short-path

apparatus.

(b) Conversion into the tetra-acetate.—— (i) A mixture of the tetrachloroglycol (4 g.), potassium acetate (15 g.) potassium iodide (0.8 g.), and acetic acid (40 c.c.) was boiled under reflux for 5 hours. Isolation of the product as in (a) gave an oil (2.5 g.). Partial decomposition occurred on high-vacuum distillation.

(ii) Silver acetate (4.6 g.) was added to a solution of the tetrachloroglycol (1 g.) in acetic acid (25 c.c.), and the mixture was boiled for 6 hours under reflux. Isolation of the product as in (a) gave an oil (0.6 g.). On this occasion the product after distillation appeared to be purer but yet failed to yield a solid.

(c) Rearrangement of the tetra-acetate.—— (i) The crude tetra-acetate (1.0 g.) was heated with acetic anhydride (1 c.c.) containing a trace of sulphuric acid for 15 minutes.

(ii) The crude tetra-acetate (0.9 g.) was heated for 1 hour with mercuric acetate (0.04 g.) and acetic anhydride (0.7 g.) and then left overnight.

In both (i) and (ii) working up in the usual way afforded a product amounting to <0.3 g., and a homogeneous fraction could not be obtained on distillation.

Reaction of sym-dichloroacetone and sodium acetylide in liquid ammonia

A solution of sodium acetylide was prepared by adding

sodium (5 g.) in small pieces to stirred liquid ammonia (250 c.c.) through which pure dry acetylene was passing. sym.-Dichloroacetone (27.6 g.) dissolved in dry ether was added dropwise to the solution. Stirring for 1½ hours with slow passage of acetylene was followed by addition of ammonium chloride (12 g.) to decompose the product. After evaporation of the ammonia, the red cake was treated with ether and with water, which partly dissolved the solid. Complete solution was effected by the addition of a little dilute sulphuric acid. Thorough extraction with ether resulted in the isolation of only 0.3 g. of material. The product is thus shown to be water- and/or acid-soluble; presumably the chloro-groups are sufficiently reactive to be attacked by the solvent ammonia. The reaction was not further examined.

Ethyl γ:γ-diethoxyacetoacetate

This ester was prepared as described by Dakin and Dudley (92) by condensation of ethyl diethoxyacetate and ethyl acetate. The product had b.p. 74-75°/0.4 mm., 103-105°/2 mm., n_D^{25} 1.4259. (Dakin and Dudley give b.p. 112-115°/7-8 mm.)

Copper salt of ethyl γ:γ-diethoxyacetoacetate

The procedure used was given by Conrad and Guthzeit (110) for the preparation of the copper salt of ethyl acetoacetate. Ethyl γ:γ-diethoxyacetoacetate (5.0 g.)

in ethanol (5 c.c.) was vigorously stirred mechanically with a solution of copper sulphate (3 g.) in water (100 c.c.) while dilute ammonia solution was added dropwise until the neutral point was reached. The copper salt separated and was filtered off, washed with water, and dried; it crystallised from light petroleum (b.p. 60-80°) in green needles (4.5 g., 78%), m.p. 111-112° (Found: C, 48.3; H, 6.9; Cu, 12.6. $C_{10}H_{17}O_5Cu_{\frac{1}{2}}$ requires C, 48.2; H, 6.9; Cu, 12.8%).

Diethyl γ:γ-diethoxyacetomalonate

The copper salt (4.5 g.) of ethyl γ:γ-diethoxyacetoacetate, ethyl chloroformate (1.9 c.c.; dried, freshly distilled), and dry benzene (4 c.c.) were boiled under reflux for 4 hours. The precipitate was filtered off and washed with benzene; the combined filtrate and washings were shaken with cooled sulphuric acid (0.2 N) and then with water, and evaporated. Fractional distillation of the residue (4.2 g.) gave ethyl γ:γ-diethoxyacetoacetate and diethyl γ:γ-diethoxyacetomalonate (0.5 g., 10%). The latter had b.p. 93-96°/0.1 mm., n_D^{20} 1.4402, but was not purified sufficiently to be analysed. The yield of crude product could be increased to 15-20% when larger quantities of ethyl chloroformate were used.

Addition of hypobromous acid to 1:4-diacetoxybut-2-ene-2-carboxylic acid

N-Bromosuccinimide (2.o g.; powdered) was added to a solution of the ethylenic acid (2.0 g.) in water (40 c.c.) containing a few c.c. of ether. After 14 hours' shaking, the mixture, now colourless and homogeneous, was saturated with sodium chloride and extracted with ether. The extract was washed with brine, dried ($MgSO_4$), and evaporated to an amber viscous syrup (2.4 g.) which was converted into the methyl ester by diazomethane in ether. The amber syrup obtained on removal of solvent was distilled (b.p. ca. $115^{\circ}/5 \times 10^{-4}$ mm., n_D^{16} 1.481-1.484) but could not be induced to crystallise.

The methyl ester (0.7 g.) was heated for $3\frac{1}{2}$ hours at 110-120° with silver acetate (0.6 g.) in acetic anhydride (4 c.c.) and the reaction mixture poured into water. The acetyl derivative, a yellow oil (0.5 g.) isolated with ether from water also would not crystallise.

1:4-Diacetoxy-2-methylbut-2-ene

This compound was prepared according to the directions of Shepard and Johnson (94) by addition of bromine in chloroform to a well-stirred solution of isoprene in chloroform kept below -25° and conversion of the crude dibromide into the diacetate by heating with fused potassium

acetate and acetic acid for 18 hours at 100°. Isolation of the product from water by means of ether, followed by distillation, retreatment with potassium acetate—acetic acid, and fractionation gave a product containing less than 2% of bromine, b.p. 127-129°/16 mm., n_D^{22} 1.4490, in 45% yield. (Shepard and Johnson give b.p. 120.5-122.5°/10 mm.; n_D^{20} 1.4494; Br, 0.27%; yield 32-40%.)

Attempted preparation of 1:4-diacetoxy-2-acetoxymethylbut--2-ene

The general procedure was as follows. 1:4-Diacetoxy-2-methylbut-2-ene (10 g. — 30 g.) was heated in carbon tetrachloride with powdered, recrystallised N-bromosuccinimide for 10-20 minutes at or below the b.p. When reaction was complete or almost complete (determined by the visible conversion of N-bromosuccinimide into succinimide) the solution was cooled, filtered, and evaporated to small bulk. The residue was heated with potassium acetate in acetic acid for 3—18 hours at or near the b.p. and then added to water; the product was isolated with ether and distilled with careful fractionation. The proportion of N-bromosuccinimide: diacetate was either 1:1, 0.85:1, or 0.5:1, and the volume of solvent was 7 x, 5 x, or 3x the volume of diacetate. The presence with the diacetate of an equal weight of acetic anhydride during bromination

(intended to hinder deacetylation) was found disadvantageous. The best conditions were very probably 1 mol. of diacetate with 0.85 mol. of N-bromosuccinimide and 3 volumes of carbon tetrachloride kept near the b.p. for 10 minutes with intermittent heating and shaking, the crude product then being refluxed for at least 6 hours with potassium acetate dissolved in acetic acid. The specimen of product which appeared from its properties to be the purest was obtained from reactions under such conditions followed by treatment with silver acetate in boiling acetic acid for 3 hours. This was a colourless liquid, b.p. 96-98°/0.2 mm., n_D^{18} 1.4618.

Ethyl 2:2-diethoxyethylmalonate

Ethyl sodiomalonate (from sodium, 14.2 g.) in ethanol (200 c.c.), and bromoacetal (80 g.) were dissolved by vigorous stirring while warm, and the reaction was effected in an autoclave under the conditions given by Perkin and Pink (96) except that the autoclave used was unstirred. Yields did not suffer as a result of this modification. The product was worked up in the same way and on distillation through a Vigreux column ethyl 2:2-diethoxyethylmalonate (71.2 g., 64%) was isolated as a liquid, b.p. 108-109°/0.7 mm., n_D^{18} 1.4282.

1:1-Diethoxy-3:3-di(hydroxymethyl)propane

Lithium aluminium hydride (9 g.) was crushed and was dissolved in dry ether (250 c.c.) by heating for 2 hours in a flask fitted with a reflux condenser and a dropping funnel and protected from atmospheric moisture. Ethyl 2:2-diethoxyethylmalonate (40 g.) in dry ether (60 c.c.) was added dropwise with frequent shaking over 1½ hours. Cooling with an ice-water bath was employed to such an extent that with this rate of addition the mixture refluxed gently. The mixture was then boiled for 2 hours, and the excess of lithium aluminium hydride was decomposed by a little ethyl acetate followed by water. Enough water (100 c.c. in all) was used to convert the resulting gelatinous white mass into a mobile slurry. After standing for some hours, the slurry was extracted with ether in a continuous-extraction apparatus for 15 hours. The ethereal solution was dried (Na_2SO_4) and evaporated. Distillation of the crude product (25.9 g.) through a short Vigreux column from a flask loosely packed with glass wool gave, after a small fore-run (2 g.) (presumably the partially reduced compound), 1:1-diethoxy-3:3-di(hydroxymethyl)propane (21.1 g., 75%) as a viscous liquid, b.p. 99-103°/5 x 10^{-4} mm., 123-125°/0.05 mm., n_D^{17} 1.4511 (Found: C, 56.2; H, 10.3. $C_9H_{20}O_4$ requires C, 56.2; H, 10.5%).

The diol did not yield a solid dibenzoate, a solid benzylidene derivative, or a bisphenylurethane; and attempts to utilise the potential aldehyde function in the preparation of solid derivatives (e.g. a 2:4-dinitrophenylhydrazone, or a diurethane) were unsuccessful.

3:3-Di(acetoxymethyl)-1:1-diethoxypropane

1:1-Diethoxy-3:3-di(hydroxymethyl)propane (20.0 g.), dry pyridine (55 c.c.), and acetic anhydride (33 c.c.) were mixed and after the initial exothermic reaction were heated on the steam-bath for 1 hour, set aside overnight at room temperature, and then added to crushed ice (100 g.). Occasional stirring in the course of several hours, latterly with ether present, hydrolysed most of the unused acetic anhydride before the mixture was extracted with ether (3 x 150 c.c.). The combined extracts, while kept below room temperature by water cooling, were shaken with successive small portions of ice-cold sulphuric acid (< 2 N) until the aqueous layer was acid to Congo red; whereupon it was immediately withdrawn and the ethereal layer was freed from acid by washing several times with sodium hydrogen carbonate solution, and finally with water. Drying (Na_2SO_4), evaporation of the solvent, and distillation through a Vigreux column gave 3:3-di(acetoxymethyl)-1:1-diethoxypropane (25.4 g., 88%), b. p. 122°/0.6 mm., 142°/2 mm., n_D^{15} 1.4352

(Found: C, 56.4; H, 8.8. $C_{13}H_{24}O_6$ requires C, 56.5; H, 8.75%).

A sample of this acetal was added to a cold saturated solution of 2:4-dinitrophenylhydrazine in aqueous acetic acid (80%). Heating at the b. p. for 10 minutes, cooling, and dilution with water gave an oil that soon solidified, and recrystallisation from benzene—light petroleum (b. p. 40-60°) gave 3:3-di(acetoxymethyl)propanal 2:4-dinitrophenylhydrazone as yellow plates, m. p. 56-57° (Found: N, 14.7. $C_{15}H_{18}O_8N_4$ requires N, 14.65%).

Bromination of 3:3-di(acetoxymethyl)-1:1-diethoxypropane

This reaction was carried out substantially in the manner of Simpson (99); equimolecular quantities of the acetal (1.69 g.), dry pyridine (0.49 g.), and bromine (0.98 g.) were mixed, the bromine being added dropwise over a few minutes to the other reagents in a swirled and occasionally water-cooled flask. The red solution was heated at 60-65° for 1 hour, and when cold the resulting brown slurry was thoroughly extracted with ether. Drying (K_2CO_3) at 0° and evaporation of the extract yielded crude 3:3-di(acetoxymethyl)-2-bromo-1:1-diethoxypropane (1.6 g., 75%) as a yellow liquid, n_D^{19} 1.4635. Decomposition occurred on distillation of the crude product. Similar

results were obtained when bromination was carried out by the reaction of N-bromosuccinimide and the acetal according to Marvel and Joncich (100).

Replacement of bromine by an acetoxy-group took place very readily to a small extent; but under moderate conditions (an excess of potassium acetate in boiling dry ethanol) not more than 15% of the bromoacetal was so converted. Under more severe conditions (e.g. heating to 180° in a sealed tube with potassium acetate—ethanol) most of the bromine was removed, but decomposition occurred and a mixture of several products (none of them identified) resulted.

Hydrolysis of 3:3-di(acetoxymethyl)-1:1-diethoxypropane by tartaric acid

(a) The acetal (2.0 g.) was stirred for 1½ hours at 0° with a solution of tartaric acid (1.0 g.) in water (10 c.c.). The suspended oil was extracted with ether, and the ethereal solution was freed from acid by washing with sodium hydrogen carbonate solution and then water, dried (Na_2SO_4), and evaporated. Unchanged starting material (1.75 g.) was recovered.

(b) The acetal (1.75 g.) was shaken continuously for 40 minutes at 20° with a solution of tartaric acid (5 g.) in water (4 c.c.). The slightly turbid mixture was worked up

as before and distillation of the product (0.9 g.) yielded a slightly impure compound (0.55 g., 55%). Further distillation gave the pure product (0.3 g.), b. p. 145°(bath)/10^{-4} mm., n_D^{21} 1.4417, which was probably 4-acetoxymethyl-2-hydroxytetrahydrofuran (Found: C, 52.85; H, 7.55. $C_7H_{12}O_4$ requires C, 52.5; H, 7.55%).

Attempted preparation of 3:3-di(acetoxymethyl)-1-acetoxy-prop-1-ene by the action of acetic anhydride—potassium acetate on 3:3-di(acetoxymethyl)-1:1-diethoxypropane

A solution of the acetal (5.0 g.) and potassium acetate (0.35 g.; crystalline) in acetic anhydride (8.3 c.c.) was heated under reflux by an oil-bath (155-160°) for 1½ hours, and then cooled. Ether was added and acid was washed out with water (4 x 2 c.c.) and sodium carbonate solution (5%; 2 x 2 c.c.). After drying (Na_2SO_4) and removal of ether and acetic anhydride, fractionation showed that the product (4.5 g.), b. p. 100-106°/0.25 mm., was the starting compound contaminated with a small amount of olefinic material.

The reaction of 3:3-di(acetoxymethyl)-1:1-diethoxypropane with acetic anhydride—ferric chloride

Ferric chloride (trace; hydrated) dissolved in a mixture of the acetal (5.0 g.) and acetic anhydride (8 c.c.) with the production of heat. The solution was kept at

50° for 3 hours, during which time it gradually darkened, and was then poured into sodium hydrogen carbonate solution (15 c.c.). Ether—extraction was followed by washing of the ethereal solution with sodium hydrogen carbonate solution (3 times) and water (3 times), drying (Na_2SO_4), and removal of solvent and acetic anhydride. The residue (4.5 g.) was fractionated. No pure compound could be obtained in significant quantity from the distillate (3.2 g.) other than unchanged starting material (1.4 g.).

Attempted preparation of 3:3-di(acetoxymethyl)-1-ethoxy-prop-1-ene by the action of acetyl chloride—pyridine on 3:3-di(acetoxymethyl)-1:1-diethoxypropane

A mixture of the acetal (5.0 g.), dry benzene (5 c.c.), dry pyridine (2.5 c.c.), and acetyl chloride (1.5 c.c.) was heated on the steam-bath for 1 hour, poured into cold water (70 c.c.), and extracted with benzene (3 x 30 c.c.). Washing with ice-cold dilute sulphuric acid, sodium hydrogen carbonate solution, and water, evaporation of benzene, and fractionation of the product (4.9 g.) yielded a distillate (4.5 g.), of which the bulk (4 g.) had b. p. 108-111°/0.3 mm., n_D^{15} 1.437, and which was obviously mainly unchanged acetal.

3:3-Di(acetoxymethyl)-1-ethoxyprop-1-ene

Sodium hydrogen sulphate (0.05 g.; fused, powdered) and a few porous chips were added to 3:3-di(acetoxymethyl)--1:1-diethoxypropane (15.4 g.) in the flask (100 c.c.) of a distillation assembly. The flask was fitted with a wide-bore, short still-head without fractionating column. With the apparatus evacuated to 0.5 mm., the flask was immersed in an oil-bath preheated to 110°, and the temperature of the latter was increased to 140° as rapidly as the copious frothing within the flask allowed. After 4 minutes' heating, distillation began and, carried out as quickly as possible (bath temperature 140-160°), was complete, except for the last traces, 5 minutes later. The tarry residue amounted to 0.5 g. Ethanol was collected in the cooled trap. The distillate (12.2 g.) contained a small quantity of starting material collected at the beginning of the distillation , and this higher-boiling liquid was separated from the product by normal fractionation, through a Vigreux column, which gave pure 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene (10.6 g., 83%), b. p. 95-97°/0.4 mm., n_D^{20} 1.4446 (Found: C, 57.0; H, 7.3. $C_{11}H_{18}O_5$ requires C, 57.4; H, 7.9%). The ultra-violet absorption indicated that only one double bond was present. Longer heating than described above caused loss of material

through elimination of acetic acid, polymerisation, and decomposition.

Treatment with a solution of 2:4-dinitrophenylhydrazine in aqueous acetic acid in the manner described above for 3:3-di(acetoxymethyl)-1:1-diethoxypropane yielded the same derivative, 3:3-di(acetoxymethyl)propanal 2:4-dinitrophenylhydrazone, as yellow plates, m. p. and mixed m. p. 56-57°.

3:3-Di(acetoxymethyl)-1-ethoxyprop-1-ene (another preparation)

3:3-Di(acetoxymethyl)-1:1-diethoxypropane (4.6 g.), acetic anhydride (8 c.c.), and p-toluenesulphonic acid (0.02 g.) were heated in a distillation apparatus for 3 hours. Keeping the oil-bath temperature below 150° allowed acetic acid, ethyl acetate, and a little acetic anhydride to distil. The black mixture was poured into cold water (10 c.c.), ether was added, and the ethereal extract was washed several times with water and then sodium carbonate solution (5%), and dried (Na_2SO_4). Removal of ether and acetic anhydride and distillation of the dark residue (3.7 g.) yielded a crude liquid (1.0 g.) from which by fractionation 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene (0.5 g., 13%) was isolated, b. p. *ca.* 85°/0.3 mm., n_D^{18} 1.4458 (Found: C, 56.6; H, 7.65%). The reaction products contained no unchanged acetal.

(±)-Cordycepose

3:3-Di(acetoxymethyl)-1-ethoxyprop-1-ene (1.00 g.) was treated with a solution of perbenzoic acid (0.60 g.; 1 mol.) in dry chloroform (34 c.c.). Titration showed that the reaction was to a large extent complete after 1 hour at 15°; the solvent was evaporated off 24 hours later after storage at 0° when 98% of the perbenzoic acid had been consumed.

The residue, consisting of the crude epoxy-ether and benzoic acid, was heated on the steam-bath for 1 hour with hydrochloric acid (2N; 25 c.c.) and ethanol (10 c.c.). On cooling, the mixture was shaken with benzene (3 x 20 c.c.) and diluted to 70 c.c. with water. Passage through a column of anion-exchange resin (Amberlite IR-4B pre-saturated with carbon dioxide) followed by evaporation to dryness under reduced pressure gave a brown syrup (0.46 g.), which was dissolved in a little methanol. The solution was filtered twice through a charcoal pad; the filtrate was evaporated to dryness; and the residue was dissolved in water (<1 c.c.), washed with ether, evaporated to dryness under reduced pressure, and dried (P_2O_5) for 18 hours at 0.5 mm. The product, (±)-cordycepose, was a colourless syrup (0.33 g., 58%).

Cordycepose p-nitrophenylosazone

The p-nitrophenylosazone was prepared from synthetic (±)-cordycepose by the method used by Bentley et al. (74) to prepare nitrophenylosazones from natural (-)-cordycepose. The sugar (42 mg.) in hydrochloric acid (2N; 2 c.c.) was treated with p-nitrophenylhydrazine (0.3 g.) in hydrochloric acid (2N; 5 c.c.). After 4 hours at room temperature a little oily solid material (8 mg.) was filtered off and discarded; red precipitates were then collected daily for 10 days, washed with water, and dried. Recrystallisation of the combined solid (55 mg.) from ethanol and nitromethane gave cordycepose p-nitrophenylosazone as a dark-red microcrystalline powder (from nitromethane), m. p. 259-260° (Kofler block). The derivative gave a blue colour with sodium hydroxide in aqueous ethanol. The m. p. was undepressed on admixture with a specimen of natural cordycepose p-nitrophenylosazone (prepared from a solution of acid-hydrolysed cordycepin). A mixture of the synthetic and naturally-derived osazones gave a single sharp band when developed in ethyl acetate on alumina.

Oxidation of 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene by Prévost's reagent

The reaction between 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene (1.0 g.) and iodine silver benzoate in benzene

was carried out by the procedure of Witcoff and Miller (16); after heating under reflux for 20 hours and working up, a yellow syrup (1.9 g.) resulted. This product, containing combined iodine, failed to yield a pure component on distillation or attempted crystallisation, and a portion (0.2 g.) was hydrolysed by heating in hydrochloric acid (2N; 2 c.c.) for 15 minutes on the steam-bath with occasional shaking. Iodine evaporated while the mixture was heated, and benzoic acid along with a little tar was removed by shaking it with benzene when cold. The aqueous acid solution was treated with p-nitrophenylhydrazine (0.5 g.) in hydrochloric acid (2 N; 10 c.c.) and the derivative was isolated as described above. The product (45 mg., crude) gave cordycepose p-nitrophenylosazone, m. p. and mixed m. p. with a specimen prepared from synthetic (±)-cordycepose 256-257° (Kofler block).

Cordycepose p-bromophenylosazone (cf. Bentley et al., ref.74)

Synthetic (±)-cordycepose (0.10 g.), p-bromophenylhydrazine hydrochloride (0.25 g.), and sodium acetate (0.30 g.) in acetic acid (15% v/v; 2.5 c.c.) were heated on the steam-bath for 1½ hours. Some bright yellow oil separated soon after mixing. The mixture was cooled and brown, oily, partly crystalline material was filtered off (127 mg., after washing with water). No product could

be isolated from the reaction mixture other than N-acetyl-N'-p-bromophenylhydrazine (78 mg., m. p. 156-160°; the collected material washed free from oily matter with the minimum quantity of ice-cold ethanol), which after charcoal treatment crystallised from water in colourless clustered blades, m. p. 162-163° (Kofler block) (Found: C, 41.8; H, 4.1; N, 12.3. Calc. for $C_8H_9ON_2Br$: C, 41.95; H, 4.0; N, 12.2; Br, 34.9%). [Bentley et al., loc. cit., report clustered blades, m. p. 163-164° (Found: C, 43.1; H, 3.8; N, 12.0; Br, 35.2. $C_{17}H_{18}O_2N_4Br_2$, cordycepose p-bromophenylosazone, requires C, 43.4; H, 3.9; N, 11.9; Br, 34.0%).] This acetyl derivative was obtained by heating p-bromophenylhydrazine (0.40 g.) and acetic acid (15% v/v; 5.5 c.c.) on the steam-bath for 1½ hours. The crude product collected on cooling (0.16 g.) yielded, after trituration with cooled ethanol and recrystallisation, the pure compound as colourless blades, m. p. and mixed m. p. with the material described above 162-163° (Kofler block).

Exactly the same results were obtained when natural cordycepose (from cordycepin, 300 mg.) was given the same treatment as the synthetic sugar.

Bromination of 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene by N-bromosuccinimide

The vinyl ethyl ether (2.0 g.) was refluxed with N-bromosuccinimide (1.5 g.) in dry carbon tetrachloride (7 c.c.) for 30 minutes. After cooling, succinimide was filtered off and the solvent was evaporated. Dry benzene was added and fitration and evaporation repeated. Attempted vacuum distillation of the residue was stopped when loss of hydrogen bromide occurred at 70°. The residue was therefore added to dry ethanol (10 c.c.) containing calcium carbonate (2 g.) and shaken for 30 minutes at room temperature; water was then added and the mixture was extracted with ether. Since analysis showed that only a small proportion (ca. 15%) of the bromine combined in the organic reaction product had been replaced, the treatment with ethanol-calcium carbonate was repeated on the extracted material; on this occasion the slurry was refluxed for 30 minutes and little further removal of bromine resulted. The organic material was isolated as before and distilled (0.02 mm.). Hydrogen bromide was evolved as distillation proceeded and it was not possible to isolate a pure product from the distillate.

Heating the product of a similar bromination reaction with silver acetate (2 g.) in dry benzene (25 c.c.)

under reflux for 2 hours brought about the replacement of 10% of the combined bromine. The residue after filtration and evaporation of the benzene was then refluxed for 15 hours with silver acetate in acetic acid to obtain complete removal of the remainder of the bromine. Distillation (10^{-4} mm.) gave an unidentified liquid. It was unsaturated, free from halogen, and soluble in water, and had n_D^{19} *ca.* 1.463.

Addition of the elements of ethyl hypobromite to 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene by treatment with bromine followed by ethanol

Bromine (2.00 g.) in light petroleum (3 c.c.; b. p. 60-80°) was added at the rate of 1 drop per second to an equimolecular weight of the vinyl ethyl ether (2.85 g.) in ether (15 c.c.) contained in a cooled (carbon dioxide—acetone) flask protected from atmospheric moisture and rotated by hand during the addition. The flask was removed from the cooling bath and after a short pause to allow some rise in temperature, the contents were added to sodium ethoxide (from sodium, 0.31 g.) in ethanol (20 c.c.) with shaking and water-cooling. After filtration from the precipitated sodium bromide, addition of ether (100 c.c.), washing with water, and drying ($MgSO_4$), distillation through a short Vigreux column gave a liquid product (1.2 g.),

n_D^{20} 1.481-1.482, which was almost certainly mainly 3-bromo-2-ethoxy-4-hydroxytetrahydrofuran.

Addition of the product of bromination by the same procedure to a well-shaken suspension of sodium hydrogen carbonate in ethanol (20 c.c.) instead of a solution of sodium ethoxide, followed by working up and distillation as above, gave two compounds, (i) 4-acetoxymethyl-3-bromo--2-ethoxytetrahydrofuran as a liquid (1.7 g., 50%), b. p. 120-121°/0.5 mm., n_D^{19} 1.4731 (Found: C, 40.8; H, 5.45. $C_9H_{15}O_4Br$ requires C, 40.5; H, 5.65%); and (ii) the slightly impure desired bromoacetal, 3:3-di(acetoxymethyl)--2-bromo-1:1-diethoxypropane (0.6 g.; n_D^{21} 1.4630).

3:3-Di(acetoxymethyl)-2-bromo-1:1-diethoxypropane

N-bromosuccinimide (4.3 g.; finely powdered, and dried over P_2O_5 for 1 hour at 50°/10^{-4} mm.) was added gradually over 20 minutes from a tube through a flexible connection to 3:3-di(acetoxymethyl)-1-ethoxyprop-1-ene (5.3 g.) in dry ethanol (20 c.c.) contained in a 2-necked flask equipped with a mechanical stirrer and protected from atmospheric moisture. During the addition and for 45 minutes thereafter, the mixture was stirred vigorously and the flask was cooled by an ice-salt bath kept at -3°. The orange solution was stirred without cooling for 2 hours, filtered from the succinimide that had crystallised, and

then in ether (150 c.c.) shaken with large volumes of sodium hydrogen carbonate solution (5 x 60 c.c.) and water until the washings were neutral. Drying ($MgSO_4$), evaporation, and rapid preliminary distillation without fractionating column (bath temperature 150-160°/10^{-5} mm.) of the colourless neutral residue (7.4 g.) gave an impure liquid (6.5 g.) Fractionation of this product using a Vigreux column, with care taken to avoid distillation of material containing free acid, yielded 3:3-di(acetoxymethyl)--2-bromo-1:1-diethoxypropane (3.9 g., 48%), b. p. 119-122°/0.35 mm., n_D^{18} 1.4630 (Found: C, 43.7, 43.4; H, 6.0, 6.3. $C_{13}H_{23}O_6Br$ requires C, 43.95; H, 6.5%). Prepared from the acetal in aqueous acetic acid, the 2:4-dinitrophenylhydrazone of the corresponding aldehyde could not be solidified.

The above reaction with N-bromosuccinimide in ethanol was unsatisfactory when carried out at temperatures above 0° or in other than anhydrous conditions. When sufficient cooling was used during mixing to maintain the reaction temperature at 20°, the above bromoacetal was obtained in poor yield (not over 15%) after separation from numerous by-products, among which were acetaldehyde, acetic acid, ethyl acetate, hydrogen bromide and 4-acetoxymethyl--3-bromo-2-ethoxytetrahydrofuran (CV, p. 82).

3:3-Di(acetoxymethyl)-1:1-diethoxyprop-2-ene

A solution of 3:3-di(acetoxymethyl)-2-bromo-1:1-diethoxypropane (5.3 g.) in dry ether (20 c.c.) was added in 1½ minutes with vigorous mechanical stirring to lithamide (from lithium, 0.5 g.; formation catalysed by ferric nitrate) in liquid ammonia (250 c.c.). Stirring of the contents of the lagged flask was continued for 30 minutes; an excess of ammonium nitrate (7 g.) was then added carefully over 5 minutes and ammonia was allowed to evaporate overnight. The remaining ammonia was removed by evaporation under reduced pressure at 30° for 3 hours, during which time the mixture was shaken frequently and treated occasionally with a little ether. After extraction with ether (4 x 30 c.c.) the residual sludge was dissolved in water; tests for bromine in the aqueous solution and in the organic reaction product showed that the action of lithamide for 30 minutes had caused the removal of at least 90% of the bromine contained in the starting compound. The ethereal extract was dried ($MgSO_4$) and evaporated; distillation of the crude product (3.6 g.) gave a main fraction of ethylenic material (2.4 g.), b. p. 88-100°/0.4 mm., n_D^{18} 1.4544-1.4595, and small amounts of higher-boiling liquid (0.5 g.) and ethyl acetate. The bulk of the main fraction was rapidly redistilled to give substantially pure 3:3-di(acetoxymethyl)-1:1-diethoxyprop-2-ene (1.5 g.,

37%), b. p. 90-93°/0.5 mm., n_D^{18} 1.4540 (Found: C, 55.3; H, 8.15. $C_{13}H_{22}O_6$ requires C, 56.9; H, 8.1%). Prolonged distillation aimed at completion of purification of the compound was instead accompanied by the evolution of more ethyl acetate with the formation of products such as the cyclic acetal (CVI, p. 84) and the hemiacetal (CVII, p. 84), presumably responsible for the unsatisfactory analytical figures.

(±)-Apiose

3:3-Di(acetoxymethyl)-1:1-diethoxyprop-2-ene (1.35 g.) was dispersed by mechanical stirring in ice-water (30 g.) in a tall beaker fitted with a thermometer and a dropping funnel. While carbon dioxide was vigorously bubbled through the mixture and stirring was continued, an aqueous solution of potassium permanganate (2%; 30 c.c.) was added dropwise, the temperature being held at 0-3° by a cooling bath until addition was complete in 50 minutes. (The end-point could not be seen because the manganese dioxide produced did not coagulate and settle until later.) The mixture was allowed to rise to 7° and a few drops of ethanol were added to destroy the excess of permanganate. Shortly afterwards, the precipitate having coagulated, carbon dioxide and stirring were stopped and most of the manganese dioxide was filtered

off. The filtrate—containing potassium hydrogen carbonate — was passed through a column of cation-exchange resin (Amberlite IRC 50) and was then extracted with ether (2 x 100 c.c.). Evaporation of the extract gave a liquid (0.2 g.). In an attempt to isolate the glycol produced by this oxidation, a portion (20 c.c.) of the clear, colourless, weakly acid (acetic), aqueous solution was evaporated to dryness — the small amount of residual syrup did not yield any crystalline material.

Hydrolysis of the remainder of the product was effected by treating the solution (200 c.c.) with concentrated hydrochloric acid (2.0 c.c.). After 24 hours at room temperature, the solution was passed through a column of anion-exchange resin (Amberlite IR-4B pre-saturated with carbon dioxide) and evaporated to dryness (<20°/0.1 mm.). The residue after washing with ether and drying was a pale yellow syrup, (±)-apiose (0.53 g., 78%).

A mixture of this product (90 mg.), p-bromophenylhydrazine (180 mg.), water (2.0 c.c.), and acetic acid (50%; 0.6 c.c.) was heated on the steam-bath. A brown oil began to separate almost immediately. After 90 minutes' heating the pale yellow supernatant liquor was decanted from the accumulated deposit while still hot; this liquor on cooling yielded a small quantity of

N-acetyl-N'-p-bromophenylhydrazine as cream-coloured blades (m. p. 159-162°). The brown product was washed with water and dissolved in ethyl acetate to remove tarry impurities. This solution was washed with water and evaporated to dryness; the residue was azeotropically dried by repeated evaporation of small volumes of benzene. The benzene-insoluble residue (N-acetyl-N'-p-bromophenylhydrazine) was filtered off and the clear reddish-brown benzene solution reduced in volume to 0.5 c.c. After 24 hours at room temperature this solution had deposited yellowish granules (41 mg.), m. p. 188-196°. Recrystallisation of some (12 mg.) of this crude product from ethanol gave apiose p-bromophenylosazone (6 mg.) as clusters of tiny, bright yellow needles, m. p. 206-208° (Kofler block) undepressed on admixture with a sample of the same m. p. derived from natural sources. The X-ray powder photographs of the synthetic and naturally-derived osazones were identical.

Unsuccessful attempts have been made to isolate (±)-apiose α-benzyl-α-phenylhydrazone. Synthetic apiose (120 mg.) was treated with α-benzyl-α-phenylhydrazine (160 mg.) in ethanol (1 c.c.) but no solid product has been obtained from the reaction mixture.

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