"PARTLY METHYLATED DERIVATIVES OF 6-DEOXY-HEXOSES".

- By -

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Thesis submitted to the University of Edinburgh
for the degree of Doctor of Philosophy.

December, 1953.

C O N T E N T S.

GENERAL INTRODUCTION.

The elucidation of the problems of carbohydrate chemistry depends on our knowledge of the simplest units. From these units, the monosaccharides, we can build up a complex molecule to include many of the familiar polysaccharides. Rapid progress has been made in the past two decades on the structure of many of these polysaccharides. Indisputable proof has been advanced that these complexes are built up of long chains of monosaccharide residues, united by glycosidic linkages. The molecules may, by this theory, exist as true chains with two terminal groups or the units may be linked together to form a loop. The chain may be an unbranched or a highly branched structure. In starch, for example, we have two polysaccharides:- amylose, un unbranched chain of α-glucopyranose units linked through the 1, 4 positions, and amylopectin, a highly bfanched structure made up of α-glucopyranose units linked as in amylose but with branched side chains joined to the main chain through the 1, 6 positions. Furthermore, these macromolecules may be built up of one type of monosaccharide, as in starch, or a number of different hexoses, pentoses and 6-deoxy hexoses may be present in a single polysaccharide. Gum arabic has been shown by Smith (1) to contain L-arabinose, L-rhamnose, D-galactose, and D-glucuronic acid residues.

The main line of attack in the elucidation of the structure of the polysaccharides has been the application of the methylation methods evolved by Purdie and Irvine (2) and by Haworth (3) and employed by Hirst in his work on cellulose, starch, glycogen and inulin (4). Essentially this work involves the complete methylation and hydrolysis of the polysacc-:haride, examination and identification of the sciss-:ion products, and isolation of the terminal units of the chains. If the polysaccharide is made up of continuous straight chains of hexoses, then a tetra-:methyl-hexose is obtained from the non-reducing end of the chain and the rest of the molecule gives rise to trimethyl-hexosds. On the other hand, a branched polysaccharide will give a tetramethyl-hexose from each non-reducing end group of each branch and from the non-reducing end group of the main chain. Trimethyl derivatives will arise from unbranched portions of the main chain and the branches, while dimethyl derivatives will be obtained from the branch points. If the polysaccharide is made up of pentos-:es or 6-deoxy-hexoses, then tri-, di-, and monomethyl derivatives will be isolated from the above portions of the molecule respectively. By the identification and characterisation of these scission products a great deal has been learnt concerning the intimate structure of the polysaccharides. Unfortunately, authentic crystalline derivatives of all the possible

fully and partially methylated derivatives of the hexoses, pentoses, and 6-deoxy-hexoses have not been synthesised and characterised, and it has not always been possible to decide in the scission products of the methylated polysaccharide which hydroxyl groups are methyl-:ated and which have been involved in union in the complex molecule.

The present work, which is concerned with the 6-deoxy-sugars, has three main aims in view:

1. The synthesis of new methyl derivatives of these sugars.

2. The preparation of ethylene oxide ring compounds from L-rhamnose (6-deoxy-L-mannose) and from L-fucose (6-deoxy-L-galactose) and the identifi-:cation of the products derived therefrom on alkaline fission.

3. The preparation of dideoxy derivatives of the 6-deoxy-sugars by reduction of the ethylene oxide ring compounds with lithium aluminium hydride.

P A R T I.

The synthesis and alkaline hydrolysis of ethylene oxide ring compounds from L-rhamnose and from L-fucose.

INTRODUCTION.

Deoxy-sugars are formally derived from ordinary sugars by the replacement of a hydroxyl group by a hydrogen atom. A number of sugars of this kind exist in Nature. Thus, 2-deoxy-D-ribose is present in many cell nuclei. Deoxy-sugars are common constituents of nucleic acids, of the cardiac glyco-:sides and of natural plant glycosides.

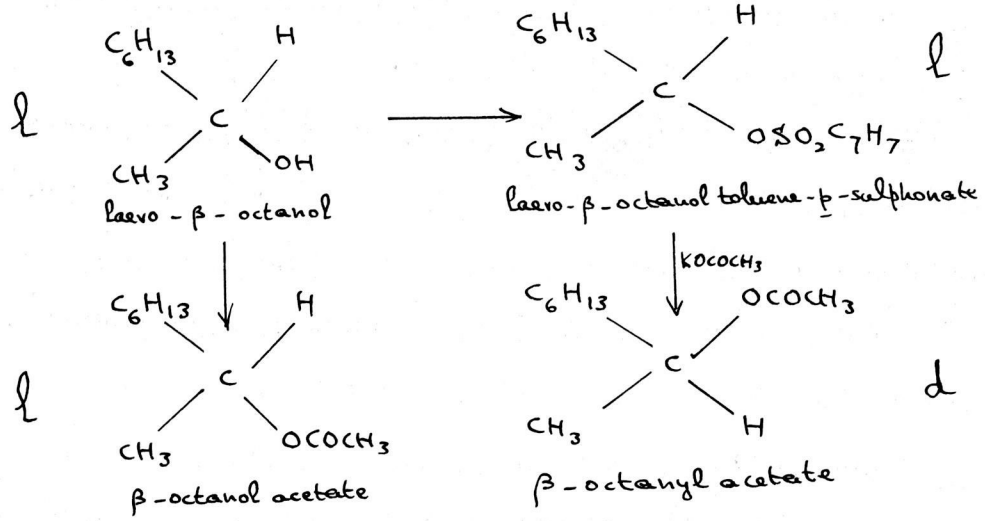
The most common deoxy-sugars are the 6- or ω-deoxy-hexoses, with a terminal methyl group in place of the primary alcoholic group. Naturally occurring 6-deoxy-sugars are 6-deoxy-D-glucose (quinovose, epirhamnose) (5), 6-deoxy-D- and -L-galactose (D- and L-fucose) (6), and 6-deoxy-L-mannose (L-rhamnose)(7). Considerable interest attaches to the two last named sugars as they are both constituents of polysaccha-:rides, and various methylated L-rhamnoses and L-fucoses have been isolated by the hydrolysis of methylated gums and mucilages. For example, 2:3:4-trimethyl-L-rhamnopyranose has been isolated from the hydrolysis products of methylated gum arabic (1) and from the somatic portion of the cells of Mycobacterium tuberculosis (8); in both of these polysaccharides L-rhamnopyranose residues have been found to occupy

terminal positions in the molecule. In addition, many mucilages such as those from mustard seed (*Brassica alba*), cress (*Lepidum sativum*) and plantain seeds contain L-rhamnose. From the mucilages of flax seed (9), slippery elm (*Ulmus fulva*) (10) and *Plantago ovata* Forsk (11) an aldobiuronic acid has been isolated and identified as 2-D-galactouronosyl-L-rhamnose, from which 3:4-dimethyl-L-rhamnose is obtained on methylation and hydrolysis. 4-Methyl-L-rhamnose has been obtained from methylated slippery elm mucilage on hydrolysis (12).

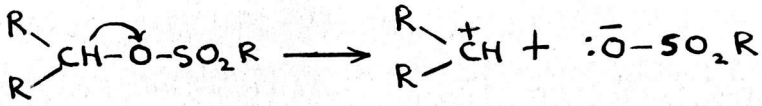
L-Fucose is the main constituent of the polysaccharide fucoidin which is found in the cell walls of some of the brown algae (Phaeophyceae). 3-Methyl- and 2:3-dimethyl-L-fucopyranose have been isolated from the hydrolysates of methylated fucoidin (13); 2:3:4-trimethyl-L-fucopyranose was obtained as a product of hydrolysis of methylated gum tragacanth (14); and L-fucose is also present in sea-urchin eggs (15), in frog spawn mucin (16) and in blood group substances (17). It has also been found that, frequently associated with alginic acid, there are polysaccharides derived from L-rhamnose residues (18) and from L-fucose residues (19), respectively. Finally, D-fucose, although not found as a constituent of polysaccharides, is a constituent sugar in the form of its 3-methyl ether of the digitalis glycosides, such as emicymarin and *iso* emicymarin.

The chemistry of the 6-deoxy-hexoses resembles that of the ordinary sugars, one of their most common reactions being esterification, and among some of the most useful esters prepared are the toluene- p - sulphonyl(20) and the methanesulphonyl(21) esters. These esters have been particularly well studied and exhibit certain unique characteristics which make them of importance in synthetic organic chemistry.

It was found by Kenyon and Phillips(22) that alkaline hydrolysis of the toluene- p-sulphonyl resi-:due of an active alcohol yields an alcohol with a different sign of rotation whereas the hydrolysis of the corresponding acetate affords an alcohol with unchanged sign of optical rotation. These authors found that laevo-β-octanol gave a β-octanol acetate which was laevorotatory, while the toluene- p-sulphon-:ate from laevo-β-octanol still had a laevo rotation; replacement of the toluene- p-sulphonyl residue by acetyl gave rise to β-octanyl acetate which was dextroroatatory.

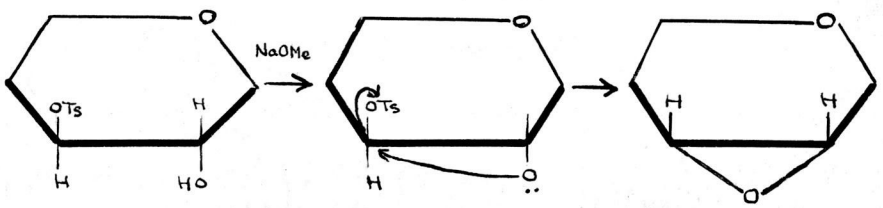


This is considered to be due to the fact that in each reaction the hydrolysis occurs at a different point; with the acetates the cleavage takes place between the acyl group and the oxygen atom (R-O ┼ Ac), while for the toluene- p-sulphonate esters it occurs between the alkyl radical and the oxygen bridge (R ┼ O-Ts), Walden inversion resulting on removal of the group attacked immediately to the asymmetric centre. Peat(23) considers that the same type of reaction occurs with the toluene- p-sulphonyl derivatives of sugars, that the saponification of the sulphonic acid involves a break between the asymmetric carbon atom and its attached oxygen with the formation of a carbonium cation:

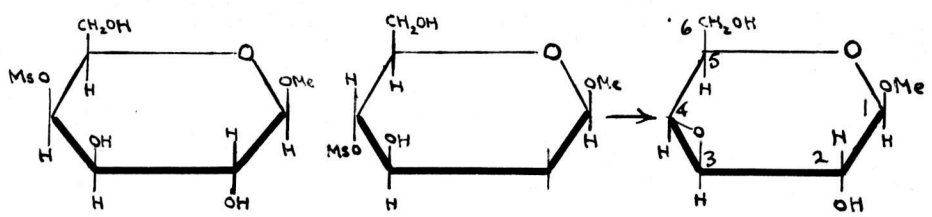


A major difference in the behaviour of the toluene- p-sulphonyl derivatives of the sugars and of the simple alcohols on hydrolysis is due to the presence and possible influence of other hydroxyl groups on neighbouring carbon atoms in the sugar chain. The actual liberation of the carbonium cation is not considered to be essential, scission must however take place between the carbon and the ester oxygen and this is followed by anhydride formation. An exchange of anions on the carbonium cation occurs, the toluene- p-sulphonyl anion being displaced by a nucleophilic group provided by a hydroxyl group present in the sugar

molecule. Proof that the replacing group comes from within the molecule is given by the treatment of a sugar toluene-p-sulphonate with sodium methoxide or with potassium acetate(24); in neither example is the toluene-p-sulphonyl residue replaced by the external ion (-OMe or -OAc): a nucleophilic group already present in the molecule effects the displace-:ment with the formation of an ethylene oxide ring. This may be illustrated as follows:-



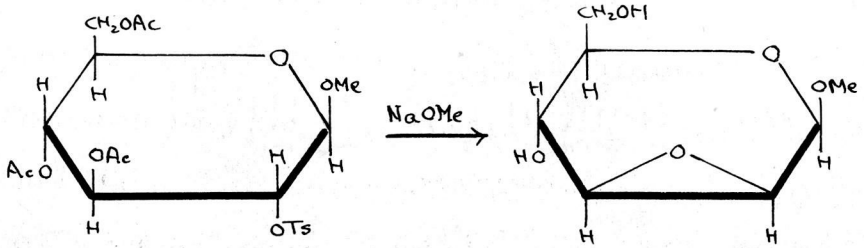
In order for ethylene oxide ring formation to take place a hydroxyl group must be present on a neighbouring carbon atom. Furthermore, this hydro-:xyl group must be trans-situated with respect to the toluene-p-sulphonyl residue. The essential charac-:ter of this trans-exchange is vividly brought out by contrasting the results of the action of sodium methoxide on methyl 4-methanesulphonyl-β-D-galacto- and -glucopyranoside:



In the D-glucoside the trans-situation of the exchanging anions on C_3 and C_4 is conducive to anhydride formation as is shown by the rapid production of methyl 3:4-anhydro-β-D-galactopyranoside; on the other hand, neither removal of the methanesulphonyl (Ms) group nor dehydration is brought about by the action of sodium methoxide on the β-D-galactoside where the adjacent exchanging anions are cis-situated, in spite of the presence of the same number of free hydroxyls as in the D-glucoside. Moreover, a four-membered anhydro ring is not formed in the D-galactoside although a trans-situated hydroxyl is available on C_2, and a freely rotating one on C_6 apparently offers no inducement to reaction. And, as with toluene-p-sulphonyl, so too with the methanesulphonyl group, there is no replacement by the external methoxyl ion.

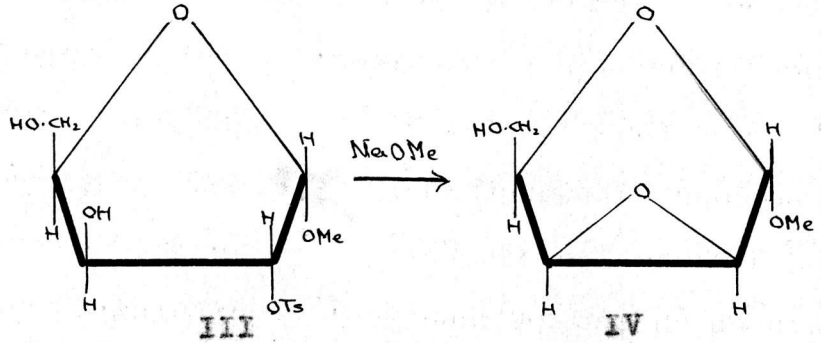
Ethylene oxide ring formation by the above mechanism is an intramolecular reaction and, as the exchange is effected by trans-disposed groups, the asymmetric centre would appear to be approached by the entering group from the opposite direction to that occupied by the sulphonic group; ring closure should then be accompanied by inversion of configuration of the carbon atom which originally bore the displaced anion. This has been verified for all cases where configurational change occurs on an

asymmetric carbon atom, where the change could be followed polarimetrically. It should perhaps be emphasised that configurational change always takes place at the asymmetric centre which originally carried the toluene-p-sulphonyl group and not at the carbon atom which carried the replacing anion. For example, methyl 3:4:6-triacetyl-β-D-glucoside 2-toluene-p-sulphonate(I) on treatment with sodium methoxide gives methyl 2:3-anhydro-β-D-manno-pyranoside(II) (26,27).



I II

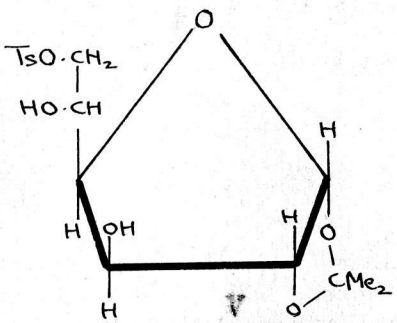
Similarly, methyl 2-toluene-p-sulphonyl-D-xylo-furanoside(III) gives methyl 2:3-anhydro-D-lyxoside (IV) (39).



III IV

Removal of the toluene-p-sulphonyl group may, however, give rise to a five-membered butylene oxide or pentaphan ring, but if the structure of the mole-:cule allows for the formation of an ethylene oxide

and a five-membered ring, the ethylene oxide ring forms preferentially. 1:2-iso Propylidene-6-toluene-P-sulphonyl-D-gluco-furanose(V) has free hydroxyl groups at C_3 and C_5 and alkaline hydrolysis gives only the 5:6-anhydride(VI) (28), but if the hydroxyl group at C_5 is blocked by another -OTs group, then the 3:6-anhydride(IX) is produced(28).



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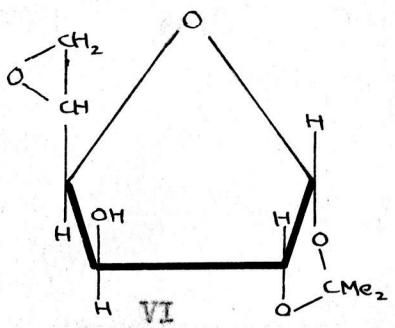
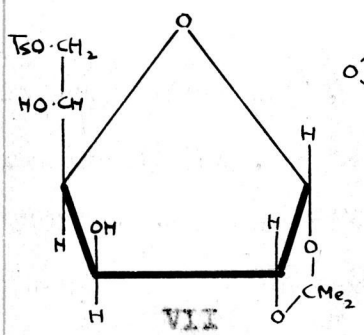


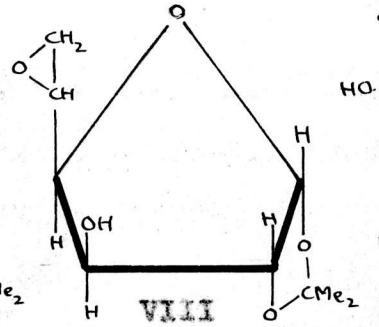
VI

It is to be observed that the formation of 3:6-anhydro rings, unlike ethylene oxide rings, does not involve any Walden inversion, but it must be remembered that C_6 is neither part of the sugar ring nor is it asymmetric. The formation of three-membered and five-membered anhydro rings is, therefore, not strictly comparable.

Sometimes the ethylene oxide ring undergoes transformation into the more stable 3:6-anhydride. Seebeck, Meyer and Reichstein(29) found that 1:2-iso-propylidene-6-toluene-P-sulphonyl-D-glucofuranose (VII) gave rise on hydrolysis to the 5:6-anhydro-derivative(VIII) which, on storage in a desiccator, was converted into the 3:6-anhydride(IX).



VII



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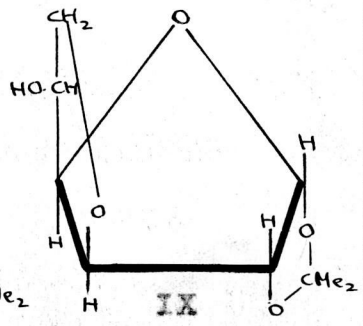
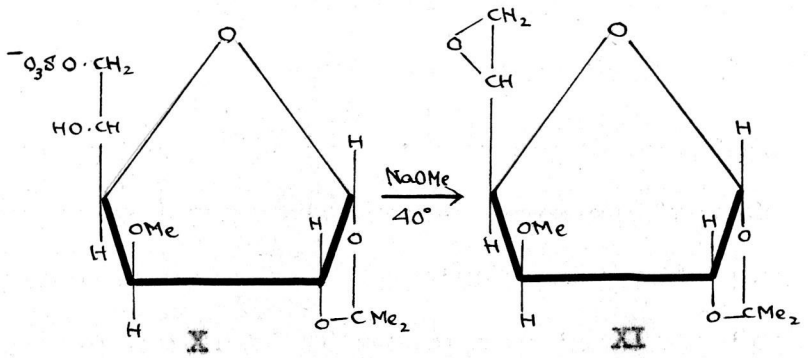


IX

If all the hydroxyl groups in the sugar sulphon-:ate are substituted by other radicals, it is very difficult to replace the toluene-p-sulphonyl group. Thus, di*iso*-propylidene-galactopyranose 6-toluene-p-sulphonate and di*iso*propylidene-glucofuranose 3-toluene-p-sulphonate(30) are hydrolysed with compar-:ative difficulty by alkali. On the other hand, methyl hexoside 6-toluene-p-sulphonates are readily converted into the methyl 3:6-anhydro-hexosides. It is significant in this connection that although the transformation of 1:2-*iso*propylidene-5:6-ditoluene-p-sulphonyl-D-glucofuranose into the 3:6-anhydro-derivative takes place easily, ring closure does not occur if the toluene-p-sulphonyl residue is on C_3 and the hydroxyl group on C_6. In the latter case hydrolysis is slow and *iso*propylidene-D-glucofuranose is the sole product(31).

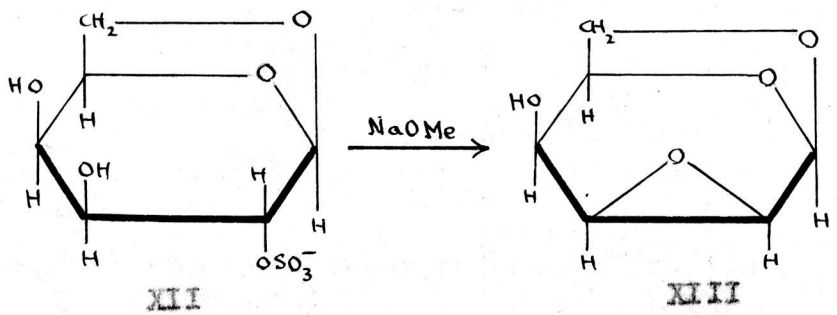
3:6-Anhydro-derivatives may also be obtained by the alkaline hydrolysis of sugar sulphates(32,33,34). If, however, the hydroxyl group on C_3 is blocked by methoxyl ion then, if the necessary conditions obtain, hydrolysis of a 6-sulphate will give rise to an ethylene oxide ring. 1:2-*iso*Propylidene-3-methyl-

glucofuranose 6-sulphate(35)(X) on treatment with sodium methoxide gave 5:6-anhydro-1:2-isopropylidene-3-methyl-glucofuranose(XI).



X XI

Although this proved that sugar sulphates can give rise to ethylene oxide ring compounds, as the sulphate was not attached to an asymmetric centre, the Walden inversion so characteristic of the hydrolysis of sulphonic esters could not be observed. However, the analogy between the two kinds of ester was completed by the isolation of 1:6-2:3-dianhydro-β-D-talopyranose(36)(XIII) from 1:6-anhydro-β-D-galactopyranose 2-sulphate(XII), inversion having occurred on the carbon atom which formerly carried the sulphate group.



XII XIII

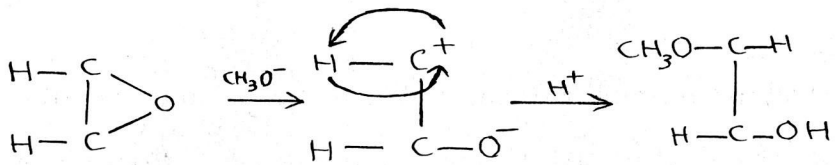
To summarise, ethylene oxide ring formation in sugars is an intramolecular reaction brought about by

a trans-exchange of anionoid groups on a potential carbonium cation, the displacing ion being anionoid oxygen derived from a hydroxyl group by proton removal. The fact that acid hydrolysis of a sugar ester is not known ever to have given rise to an anhydro ring is experimental corroboration of this view since, if it be correct, it is obvious that hydrolysis of a sugar ester leading to the elimination of the elements of water can only be accomplished by alkaline reagents. If the ester group is situated on C_6 and there is a free hydroxyl group on C_3, alkaline hydrolysis will give rise to a 3:6-anhydro-derivative which may be preceded by 5:6-ethylene oxide ring formation if the appropriate conditions obtain.

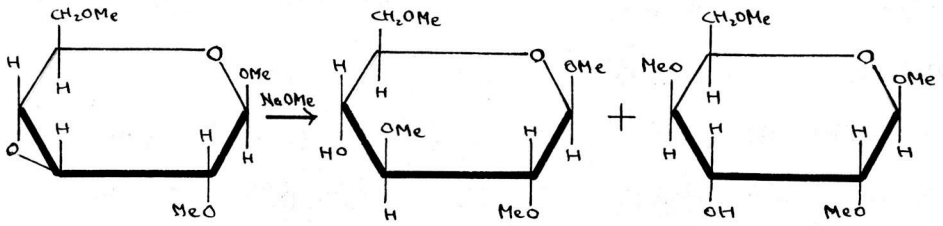
The behaviour of the ethylene oxide ring and the 3:6-anhydro ring towards hydrolytic reagents is quite different. The normal hydrofuranol ring is unattacked by acid or alkali, whereas the ethylene oxide ring is easily ruptured by both acid and alkali. It is considered moreover that the same essential mechanism is involved in the scission of the ring with either type of reagent. We will consider first the effect of the alkaline reagent, sodium methoxide.

The active agent in this case is the methoxyl anion CH_3O^- and here the external ion becomes part of the final product. In other words the reaction is intermolecular in contrast with the hydrolysis of

the ester grouping which was intramolecular. The first step is for a carbonium cation to be produced at one of the carbon centres and the oxygen atom becomes negatively charged.

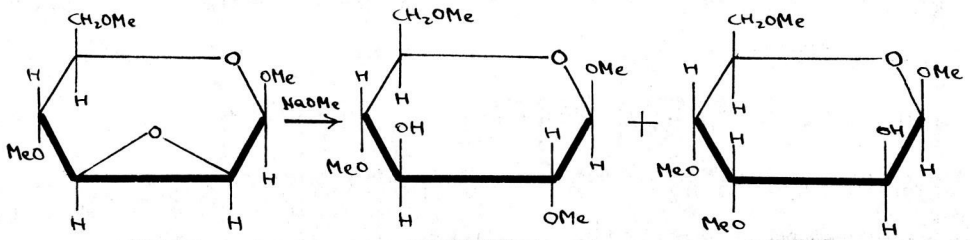


The methoxyl group can then approach the carbonium cation on the side opposite from that originally occupied by the ethylene oxide ring, union occurs and this results in a change of configuration on the car-:bon atom which acquires the methoxyl group. There are, however, two centres at which anion exchange can occur and depending on which centre develops cation activity so the final product is different. Since in many examples both centres become active, two differ-:ent sugars are produced on alkaline scission of an ethylene oxide ring and both of these are configurat-:ionally different from the ring compound itself. This can be illustrated best by specific examples: Methyl 3:4-anhydro-2:6-dimethyl-β-D-alloside(XIV) gives a mixture of methyl 2:3:6-trimethyl-β-D-gluco-:side(XV) and methyl 2:4:6-trimethyl-β-D-guloside (XVI) in the proportions of 2 : 1 (23).



XIV XV XVI

Methyl 2:3-anhydro-4:6-dimethyl-β-D-mannopyranoside (XVII) on scission with sodium methoxide gives equimolecular quantities of methyl 2:4:6-trimethyl-β-D-glucopyranoside(XVIII) and methyl 3:4:6-trimethyl-β-D-altropyranoside(XIX) (27).

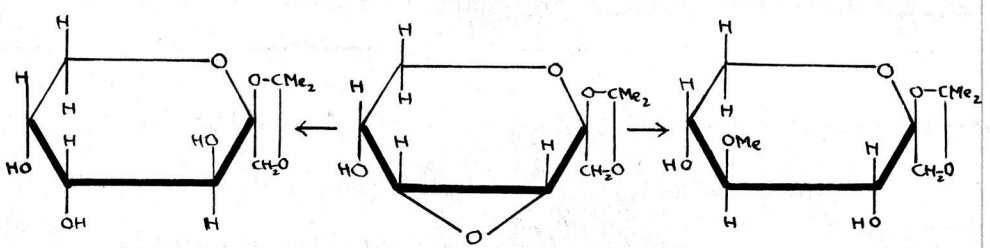


XVII XVIII XIX

If sodium hydroxide instead of sodium methoxide is used as the hydrolytic agent, a mixture of methyl 4:6-dimethyl-β-D-glucoside and -altroside is produced(37). From these examples it would appear that cleavage of the anhydro ring on either side of the oxygen atom takes place with equal facility.

It should be pointed out, however, that two products cannot invariably be recognised, the chances of the ethylene oxide ring breaking in a particular way depending presumably on steric factors. 3:4-Anhydro-1:2-isopropylidene-D-psicose (allulose) (XX) when treated with sodium hydroxide yields a mixture

of products(38) among which 1:2-isopropylidene D-fructose(XXI) was detected, inversion having occurred at C_3. With sodium methoxide however, inversion was mainly on C_4 and 4-methyl-1:2-isopropylidene-D-sorbose (XXII) was the chief product.



XXI XX XXII

Percival and Zobrist found(39) that hydrolysis of methyl 2:3-anhydro-D-lyxoside with sodium methoxide gave two products: 2-methyl-D-xylose and 3-methyl-D-arabinose in the ratio of 1:2; whereas, if the C_5 hydroxyl group was methylated prior to the scission of the oxide ring, then the sole product was 3:5-dimethyl-D-arabinose.

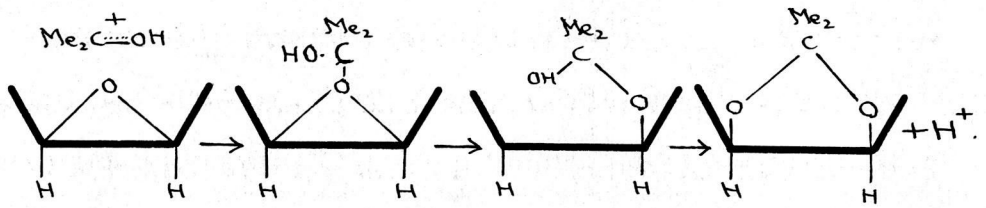
In the ring opening of methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside(XXV) with sodium methoxide Peat and Wiggins(40) obtained methyl 4:6-benzylidene-2-methylaltroside(XXVI) as the main product while the second derivative methyl 4:6-benzylidene-3-methyl-:glucoside(XXVII) was isolated only in 10% yield; further, in the case of methyl 2:3-anhydro-4:6-benzylidene-β-D-taloside Wiggins(41) only succeeded in isolating the second product, methyl 4:6-benzylid-:ene-2-methyl-β-D-galactoside, with great difficulty

and in minute yield.

Although the behaviour of ethylene oxide rings towards alkaline reagents has been most exhaustively studied, a certain amount of work has been carried out with acidic reagents and the examples so far recorded indicate that the mechanism of the reaction is essentially the same as with alkali. Thus, by the action of aqueous sulphuric acid on methyl 3:4-anhydro-β-D-galactoside derivatives of D-glucose and D-gulose are obtained(42). Mention should be made however of a result obtained by Oldham and Robertson (30) which does not follow this general mechanism. These authors failed to isolate any derivatives of glucose from the mixture obtained from the action of cold, dry hydrogen chloride in acetone on methyl 2-acetyl-3:4-anhydro-6-trityl-α-D-galactoside, but obtained instead derivatives of gulose and galactose, the latter having arisen apparently without any inversion at the asymmetric centre. As far as the writer is aware, this is the only recorded exception to the rule that ethylene oxide ring scission involves the trans-exchange of anionoid groups.

This work has been repeated recently by Labaton and Newth(51) who found that if aqueous hydrochloric acid is used as the hydrolytic agent then the reaction follows the normal trans-anion exchange and methyl 3-chloro-3-deoxy-α-D-gulosside and methyl 4-chloro-4-deoxy-α-D-glucoside are obtained. At the same time

however these authors confirmed the results of Oldham and Robertson. They found that if dry hydro-:gen chloride in acetone was employed, then methyl 3:4-<u>iso</u>propylidene-α-D-galactoside was obtained in place of the glucoside derivative and suggested that the following scheme would account for its formation:



The transformation of an anhydro-sugar into an amino-deoxy-sugar by the action of dry ammonia(40,99) is regarded by Peat(23) as an exchange of the NH_2^- anion derived from ammonia as follows:

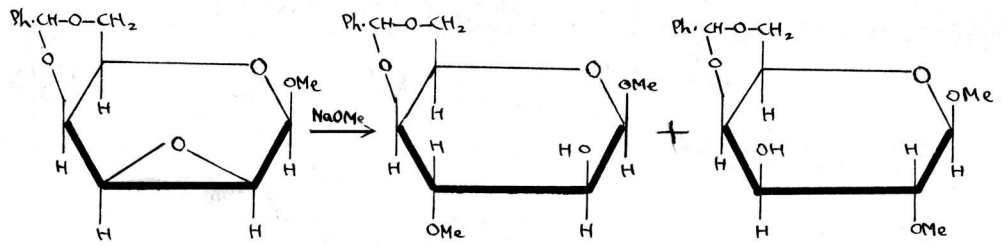
$$NH_3 \rightleftharpoons H^+ + NH_2^-.$$

No essential difference has been found in the mode of scission of the anhydro ring when ammonia is employed.

Apart from alkaline and acidic reagents, scission of the oxide ring in anhydro-sugars can be brought about by Grignard reagents(43,44,45,46). It has been found(47) that the attack of a Grignard reagent follows essentially the same course as when acid or alkaline reagents are employed, methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside giving with methyl magnes-:ium iodide methyl 4:6-benzylidene-3-deoxy-3-iodo-α-D-glucoside in 80% yield. It will be remembered that the action of sodium methoxide on this compound

gave the other scission product, methyl 4:6-benzyli-:dene-2-methylaltroside, in 90% yield while the 3-methylglucoside derivative was only obtained in 10% yield. Methyl 2:3-anhydro-4:6-dimethylalloside with methyl magnesium iodide gave a mixture of products including the 2-iodoaltroside and the 3-iodoglucoside derivatives.

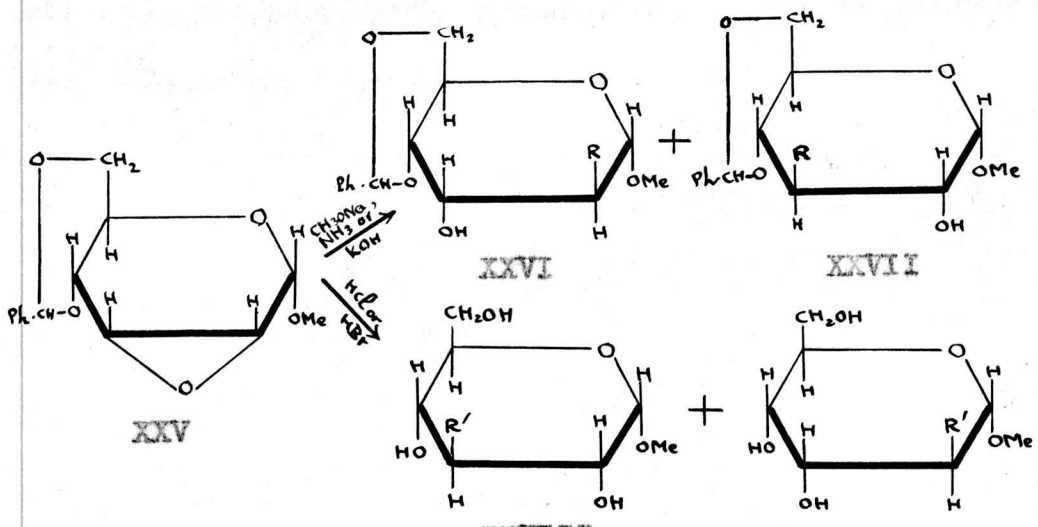
Various theories have been advanced to account for these results. Newth, Overend and Wiggins(46) came to the conclusion that if the ethylene oxide ring lies above the plane of the sugar ring in the D-series, then the C-O bond further from the glycosidic group suffers the most intensive cleavage, whereas if the anhydro ring is situated below the plane of the sugar ring then it is the C-O bond nearer to the glycosidic group which breaks to the greater extent. This is supported by the cleavage of methyl 2:3-anhydro-4:6-benzylidene-β-D-talaside(XXIII) with sodium methoxide which gave mainly the 3-methyl derivative(XXIV), a product obtained by scission of the C-O bond further from the glycosidic group.



XXIII XXIV

Similarly with methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside(40) (oxide ring below the sugar ring plane) the bond nearer the glycosidic group mainly breaks. The presence of a 1:6-anhydro ring in the molecule, however, appears to cause the opposite effect and 1:6-2:3-dianhydro-β-D-talopyranose (ring above) (49) gave mainly 1:6-anhydro-2-methyl-D-galactose, the oxide ring having broken on the side nearer to C_1.

However, acidic hydrolytic reagents according to these authors reverse the proportions of the products obtained and when acid hydrolysis occurs and the oxide ring is below the sugar ring, then it is the bond farther from C_1 which is mainly broken. The example quoted is methyl 2:3-anhydro-α-D-alloside(XXV): alkaline reagents(40) gave mainly the altroside derivative(XXVI), whereas from the action of hydrobromic and hydrochloric acids the methyl 3-chloro-3-deoxy- and methyl 3-bromo-3-deoxy-α-D-glucosides(XXVIII) were the main products.



Experiments with sulphuric and oxalic acid as hydrolytic reagents on methyl 2:3-anhydro-∝-D-alloside produce a similar effect to that of the halogen acid.

It is surprising to the writer that the position of the glycosidic group relative to the position of the ethylene oxide ring is not the determining factor in the ring fission, but in the examples quoted(XXIII) and (XXV) in one case the glycosidic methoxyl and the ethylene oxide ring are both above the plane of the sugar ring and the main product is the 3-derivative, whereas in the second case the oxide ring and the glycosidic methoxyl are again both on the same side of the sugar ring and the main product is the 2-derivative. It would appear from this that it is the relative position of the terminal carbon atom which influences the cleavage of the oxide ring.

More recent work(50) has shown that the conclusions advanced by Newth, Overend and Wiggins(48) with regard to ring cleavage cannot be applied universally. Dibenzyl hydrogen phosphate(52) reacts with methyl 2:3-anhydro-4:6-benzylidene-∝-D-allopyranoside to give (after removal of the benzyl and benzylidene groups) predominantly methyl ∝-D-altroside 2-phosphate which is what would have been expected from alkaline rather than acidic fission. Again, the action of hydrochloric acid on methyl 2:3-anhydro-4:6-benzylidene-∝-D-mannoside(53) followed the same course as alkaline fission(40,54) of the anhydro ring, and gave rise to methyl 3-chloro-3-deoxy-∝-D-

altroside in 92% yield.

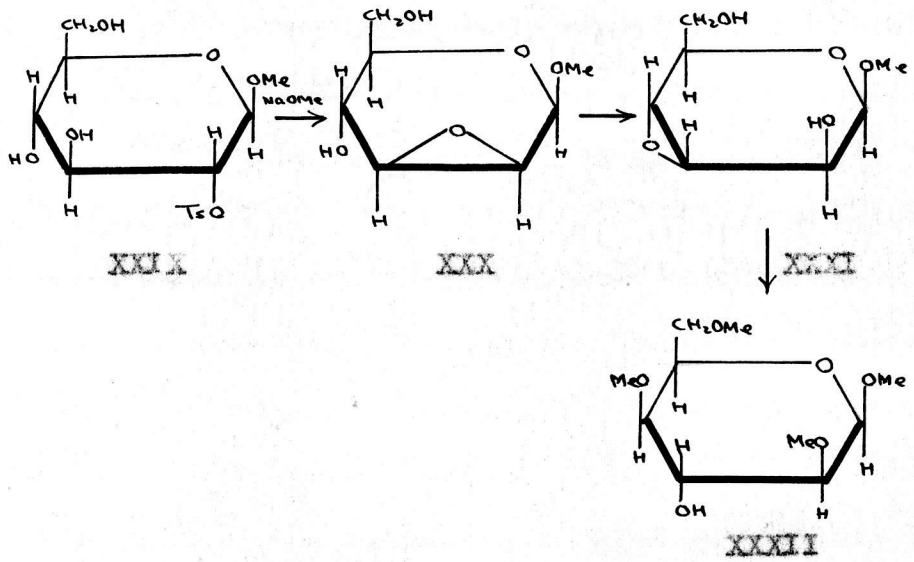
Finally an attempt has been made to extend Fürst and Plattner's rule, that "steroid" epoxides break to give a product with the two groups in polar positions (55), to sugar epoxide derivatives, and Newth and Homer (53) contend that derivatives which have the ethylene oxide ring stabilised by a 1:6-anhydro or a 4:6-benzylidene group obey this rule. The examples they cite in support of this behaviour are 1:6-2:3- and 1:6 -3:4 -dianhydro-β-D-talopyranose with ammonia(49) and the predominance of methyl 4:6-benzylidene-α-D-altroside derivatives from both methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside and methyl 2:3-anhydro-4:6-benzylidene-α-D-mannoside(53). Should the stabilising group (1:6-anhydro ring or 4:6-benzylidene group) be absent or be removed from the anhydro-sugar during reaction with acidic reagents, these authors consider it is more difficult to predict which product will be obtained in greater quantity.

Bose, Chaudhuri and Bhattacharyya(103) have examined this rule further. They support Newth and Homer in so far that they are of the opinion that the scission of the epoxide ring in 2:3-anhydro sugars by alkoxides, alkalis, ammonia and thiols gives predominantly products which have the entering groups in the polar conformation: altrose derivatives are obtained in preference to glucose derivatives from 2:3-anhydroallose and 2:3-anhydromannose derivatives; idose

derivatives are produced in preference to galactose derivatives from 2:3-anhydrogulose and 2:3-anhydrotalose derivatives; but they differ from the latter authors in that they do not consider the presence of a stabilising group necessary. When 3:4-anhydro derivatives are subjected to scission, Bose et al. consider that in the main derivatives with the entering groups in the equatorial position are obtained but that the real governing factor in these derivatives is the bulky primary hydroxyl group which must be trans to the substituent at position 4. The weakness of this theory of polar or equatorial substitution lies in the assumption that the sugar ring in all these derivatives has the Sachse trans conformation. Although there is some evidence from X-ray studies(104) that sugars in the crystalline state do exist in this form, no definite evidence has so far been advanced concerning the conformation of the sugar ring in solution. It should be emphasised that fission of the anhydro ring and substitution of the entering groups always takes place in solution and the above arguments therefore do not appear entirely conclusive to the writer.

Certain experimental results can only be explained by postulating the occurrence of multiple formation and scission of anhydro rings. The conversion of methyl 2-toluene-p-sulphonyl-β-D-glucoside(XXIX) into a D-idose derivative by Lake and Peat(58) requires inversion of configuration on C_2, C_3 and C_4 which,

the authors considered, involves the formation of a 2:3-anhydro ring followed by a 3:4-anhydro ring. The first product of the reaction of sodium methoxide is methyl 2:3-anhydro-β-D-mannoside(XXX). Excess sodium methoxide causes fission of the 2:3-anhydro ring and subsequent rearrangement leads to the formation of methyl 3:4-anhydro-β D-altropyranoside(XXXI) which is isolated as syrupy methyl 3:4-anhydro-2:6-dimethyl-β-D-altropyranoside. Further treatment of this syrup with sodium methoxide gives mainly methyl 2:4:6-trimethyl-β-D-idopyranoside(XXXII).



XXIX XXX XXXI XXXII

Levene and Compton(59) believe that formation and cleavage of a 4:5-ethylene oxide and a 1:5-anhydro ring compound (the former having D-gulose and the latter D-allose configuration) is necessary to account for the formation of methyl 6-deoxy-2:3-isopropylidene-D-allofuranoside by the alkaline hydrolysis of 2:3-isopropylidene-5-toluene-p-sulphonyl-L-rhamnofuranose. No anhydro-sugars could be isolated.

Although anhydro-sugars of the ethylene oxide type have never been detected as constituents of living material, it is tempting to visualise metabolic processes taking place in this manner and it may well be that biological interconversions of sugars are brought about by anion exchange on carbonium cations. Peat(60) in fact suggests that the change from glucose to galactose which takes place so readily in the mammary gland may be through the formation of the D-glucose 5-phosphate followed by successive intramolecular anion exchange on C_5 and C_4. Although no direct support has been found for the suggestion, it is plausible to envisage the interconversion of sugars in seaweeds and other living systems containing polysaccharide sulphates by means of ethereal sulphates and ethylene oxide ring compounds.

Apart however from the hypothetical use of such substances in Nature, the value of ethylene oxide ring derivatives in the laboratory for the synthesis of the rarer sugars has been immense and it is only necessary to examine the examples cited in this account to realise the wide variety of sugars and their derivatives which may be obtained from these ring compounds.

It was considered that the isolation of new derivatives of 6-deoxy-sugars might be achieved by the application of these methods; accordingly, methyl 4-toluene-p-sulphonyl-α-L-rhamnopyranoside and methyl 2-toluene-p-sulphonyl-α-L-fucopyranoside have been

prepared. Removal of the toluene-p-sulphonyl groups gave crystalline methyl 3:4-anhydro- and crystalline methyl 2:3-anhydro-α-L-taloside, respectively. Both these substances have been subjected to alkaline fission with sodium methoxide both before and after methylation, and attempts have been made to identify and characterise the mono- and dimethyl sugars obtained.

E X P E R I M E N T A L

All evaporations were carried out under diminished pressure, the temperature not exceeding 45°.

Carbon, hydrogen and nitrogen micro-determinations were carried out by Drs. Weiler and Strauss of Oxford.

Methoxyl group and sulphur micro-determinations were carried out by the author.

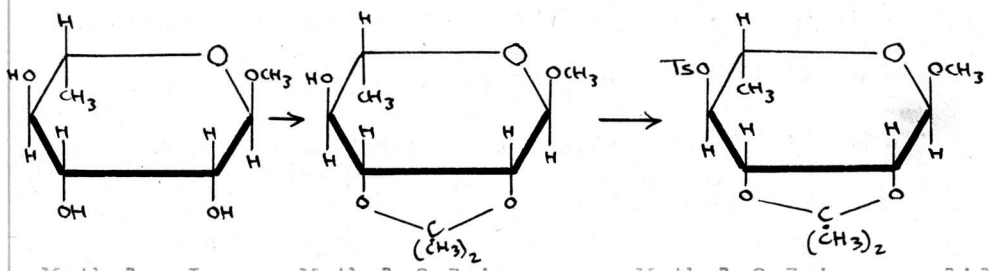
Unless otherwise stated the following solvents have been used throughout as eluants (top layer) in chromatographic analysis:
Solvent (I): n-Butanol-Ethanol-Water (4:1:5.v/v)
Solvent (II): Benzene-Ethanol-Water(169:47:15.v/v)
The following R_G values were determined:

Substance	Solvent (I)	Solvent (II)
1. L-Rhamnose	0.40	
2. 4-Methyl-L-Rhamnose	0.66	
3. 2,4-Dimethyl-L-Rhamnose	0.86	0.94
4. 2:3-Dimethyl-L-Rhamnose	0.82	0.89
5. 2:3.4-Trimethyl-L-Rhamnose	1.01	
6. Methyl 3:4-Anhydro-6-deoxy-α-L-Taloside	0.92*	
7. Methyl 3:4-Anhydro-2-methyl-6-deoxy-α-L-Taloside	1.03*	
8. 6-Deoxy-L-Idose	0.50	
9. 6-Deoxy-3-Methyl-L-Idose	0.72	
10. L-Fucose	0.21	
11. 2-Methyl-L-Fucose	0.59	
12. 2:4Dimethyl-L-Fucose	0.80	
13. 2:3:4:-Trimethyl-L-Fucose	0.94	

* A saturated aqueous solution of aniline oxalate was used as a spray, except for substances 6 and 7 where a 5% phosphoric acid solution in saturated aqueous aniline oxalate was used.

The L-fucose used for the preparation of anhydrosugars in Part II was made available to the writer through the generosity of the Institute of Seaweed Research.

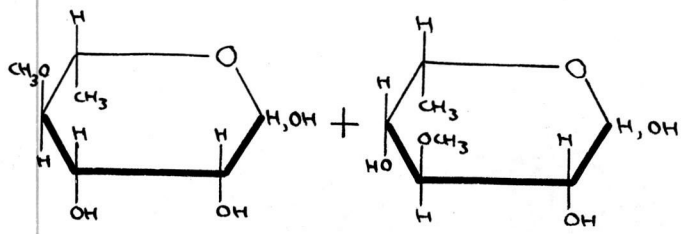
SYNTHESIS OF 4-METHYL-6-DEOXY-L-MANNOSE (4-METHYL-L-RHAMNOSE), 3-METHYL-6-DEOXY-L-IDOSE AND OF 2:4-DIMETHYL-L-RHAMNOSE.



Methyl α-L-rhamnoside.

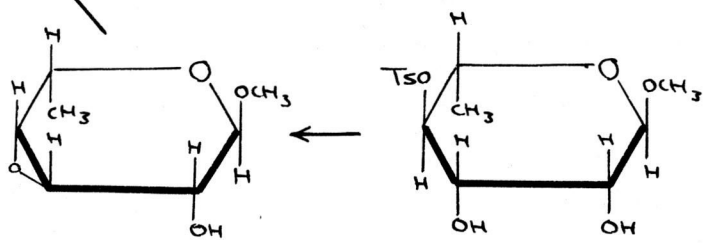
Methyl 2:3-isopropylidene α-L-rhamnoside.

Methyl 2:3-isopropylidene 4-toluene-p-sulphonyl-α-L-rhamnoside.



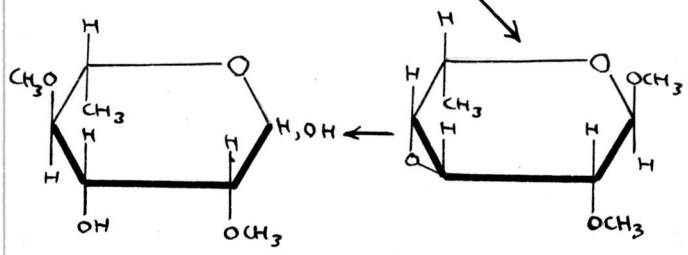
4-Methyl-L-rhamnose.

3-Methyl-6-deoxy-L-idose.



Methyl 3:4-anhydro-6-deoxy-α-L-taloside.

Methyl 4-toluene-p-sulphonyl α-L-rhamnoside.



2:4-Dimethyl-L-rhamnose

Methyl 3:4-anhydro-6-deoxy 2-methyl-α-L-taloside.

Methyl ∝-L-Rhamnoside.

∝-L-Rhamnose H_2O(25.0g.) mp.93-94°, $[\alpha]_D^{20°}$ -9°→+8° (c, 4.0 in water) was treated six times with alcohol and benzene(6:4,v/v) and gave anhydrous rhamnose(22.1g.) m.p.120°, $[\alpha]_D^{18°}$ +38°→+9°(c,2.5 in water). The latter was dissolved in methanolic hydrogen chloride solution (280c.c.;0.25%) and heated at 80° under reflux until non-reducing to Fehling's solution(40 hours). After cooling, the solution was neutralised with silver carbonate; the silver salts were removed by filtration and extracted with hot chloroform(4 x 30c.c.). The combined extracts and filtrate were evaporated under diminished pressure and gave a colourless syrup (23.85g.) which crystallised spontaneously, mp.109-110°, $[\alpha]_D^{18°}$ -62° (c,1.0 in water); lattice constants (from layer line spacings, kindness of Dr.C.A.Beevers) a = 8.1, b = 13.2, c = 7.4 (Braekken, Koren and Sorensen(106) record a = 8.2, b = 13.1, c = 7.5 for methyl ∝-L-rhamnoside).

In a second experiment, anhydrous rhamnose(21.9g.) was converted into the glycoside(23.51g.) by refluxing the sugar with a 2% methanolic hydrogen chloride solution(150c.c.) until non-reducing to Fehling's solution (6 hours).

In a third experiment, anhydrous rhamnose(3.0g.) was dissolved in dry methanol(30c.c.) and heated under reflux at 80° in the presence of an equal weight of cation exchange resin (Amberlite IR-100-H) until

non-reducing to Fehling's solution (9-10 hours). The resin was removed by filtration and washed with hot methanol (6 x 10c.c.). Removal of the solvent from the combined extracts and filtrate yielded crystalline methyl α-L-rhamnoside (2.50g.).

Methyl 2:3-isoPropylidene-α-L-rhamnoside.

This method follows very closely that of Percival and Percival(107). Methyl α-L-rhamnoside(23.50g.) was dissolved in dry acetone(1350c.c.), 20 drops of acetaldehyde and anhydrous copper sulphate(320g.) were added and the mixture shaken vigorously for 120 hours. At the end of this period, 7 drops of concentrated sulphuric acid were added and the shaking continued for a further 20hours. The copper sulphate was filtered and extracted with dry acetone(6 x 300c.c.). The filtrate and extracts were combined and neutralised with barium carbonate; the barium salts were also extracted with acetone (3 x 100c.c.). The combined extracts and filtrate were then taken to dryness and the resulting syrup distilled in the presence of a trace of barium carbonate at 110-115°(bath temperature) /0.05mm.; yield 23.61g. (75% of theory); $n_D^{16°}$ 1.4563; $[\alpha]_D^{12°}$ -14° (c, 1.0 in acetone).

In repetitions of this preparation it was found unnecessary to add concentrated sulphuric acid, as the addition of acid catalyst did not appreciably increase the yield.

Methyl

Methyl 2:3-isoPropylidene-4-toluene-p-sulphonyl-∝-L-rhamnoside.

Methyl 2:3-isopropylidene-∝-L-rhamnoside(23.0g.) in two half-lots, was dissolved in two portions of pure, dry pyridine(55c.c.) and each half was treated with finely powdered toluene-p-sulphonyl chloride(21.0g.) in small portions, with cooling and frequent shaking. After standing at 15° for 40 hours and at 22° for a further 8 hours, the dark red solutions, in which feathery crystals of pyridine hydrochloride had appeared, were poured onto finely crushed ice with stirring. A small quantity of crystals was obtained, but the main product was a sticky pink solid. Complete removal of the pyridine by repeated washing with water, however, gave a powdery amorphous solid. The first two litres of the washings were extracted with chloroform (4 x 300c.c./500c.c. washings); the extracts were successively washed with sulphuric acid solution (10%; 4 x 300c.c.), saturated aqueous sodium hydrogen carbonate solution (3 x 300c.c.), and with water (2 x 300c.c.). The combined washed chloroform extracts were dried over anhydrous sodium sulphate; removal of the solvent afforded a brown mobile syrup contaminated with pyridine. This syrup was poured onto crushed ice, and, after repeated washing to free it from pyridine, a white amorphous powder was obtained. This was combined with the main product (Total yield 28.0g.) and recrystallised from methanol in the

form of large plates(25.1g.) mp.60-61°, $[\alpha]_D^{15°}$ +22° (c, 2.9 in methanol).

Found: C, 54.64; H, 6.46. Calc. for $C_{17}H_{24}O_7S$: C, 54.84; H, 6.45%.

Methyl 4-Toluene-p-sulphonyl-α-L-rhamnoside.

Crystalline methyl 2:3-isopropylidene-4-toluene-p-sulphonyl-α-L-rhamnoside(24.0g.) was treated with methanolic hydrogen chloride(185c.c.;1%) at 70° for 75 minutes. After neutralisation with silver carbonate and extraction of the silver residues with hot methanol(4 x 50c.c.), removal of the solvents from the combined filtrate and extracts afforded methyl 4-toluene-p-sulphonyl-α-L-rhamnoside(20.2g.) as a non-reducing syrup which on hydrolysis with acid gave a negative iodoform test for acetone. It had $n_D^{12°}$ 1.5208; $[\alpha]_D^{15°}$ -74° (c, 1.4 in chloroform).

Found: S, 8.8; OMe, 8.2. Calc. for $C_{14}H_{20}O_7S$: S, 9.6; OMe, 9.3%

Methyl 3:4 Anhydro-6-deoxy-α-L-taloside.

Methyl 4-toluene-p-sulphonyl-α-L-rhamnoside (19.80g.) was dissolved in absolute alcohol(50c.c.) and, after addition of 2 drops of phenolphthalein, the solution was titrated at 75° with sodium hydroxide solution(2M; 30.0c.c.) until permanently pink; it was then refluxed at 70° for 60 minutes and allowed to stand for 18 hours at 15°. In repetitions of this experiment the pink colour was found to have faded on occasion, after the solution had been kept at 15° for 18 hours; in such cases more 2M-sodium hydroxide

solution was added until the solution became permanently alkaline. Sodium toluene-p-sulphonate was often deposited, either as large white crystals or as an amorphous powder, and was removed by filtration. Removal of the solvent under reduced pressure gave a crystalline solid admixed with syrup; which was extracted with dry ethyl acetate in the cold(5 x 20c.c.) and at the boiling point(20c.c.). Removal of the insoluble sodium toluene-p-sulphonate by filtration was followed by evaporation of the filtrate (combined extracts) yielding a semi-crystalline mass. This was recrystallised from warm light petroleum (b.p.40-60°) giving methyl 3:4-anhydro-6-deoxy-α-L-taloside (6.77g.) mp. 67-68°, $[\alpha]_D^{15°}$ -109° (c, 0.9 in water).

Found: C, 52.7; H, 7.4. $C_7H_{12}O_4$ requires C, 52.5; H, 7.5%

Chromatographic analysis with butanol:ethanol: water(4:1:5 v/v) as eluant and a 4% solution of phosphoric acid in aniline oxalate as spray gave a single spot R_G 0.92. Repeated treatment of the mother liquors with light petroleum(b.p.40-60°) brought about almost complete crystallisation and the remaining small quantity of syrup (X) (0.3g.) produced on heavy spotting on the paper chromatogram a single spot, R_G 0.92 (with above eluant and 4% phosphoric acid-aniline oxalate spray) identical with that given by the crystals.

In a subsequent synthesis of this compound starting from the same quantity of rhamnose H_2O(25.0g.)

improved yields were obtained at all intermediate stages, the yield of methyl 3:4-anhydro-6-deoxy- α-L-taloside being 8.0g., mp.68°, $[\alpha]_D^{16°}$ -110°(c, 1.1 in water).

Hydrolysis of Methyl 3:4-Anhydro-6-deoxy- α-L-taloside with N-Sulphuric Acid.

Methyl 3:4-anhydro-6-deoxy- α-L-taloside (0.05g.) $[\alpha]_D^{15°}$ -109° was boiled with N-sulphuric acid(12c.c.) until the rotation was constant (3 hours; $[\alpha]_D^{16°} \pm 0°$). This gave a reducing syrup which showed on a chromatogram a distinct spot identical with that given by authentic rhamnose (R_G 0.40) and a faint spot R_G 0.50 thought to correspond with 6-deoxyidose. On standing this syrup partially crystallised. The crystals had mp.89° alone and on admixture with rhamnose (mp. 92°).

Alkaline Hydrolysis of Methyl 3:4-Anhydro-6-deoxy- α-L-taloside with

(a) Potassium Hydroxide.

Crystalline methyl 3:4-anhydro-L-taloside(0.2g.) mp.68° was dissolved in aqueous potassium hydroxide (5%;20c.c.)(40) and the solution heated under reflux on a boiling water-bath for 20 hours. Some darkening occurred so that changes in rotation could not be followed. The cooled solution was saturated with potassium bicarbonate; the whole being then extracted twice with chloroform. The dried chloroform extracts (Na_2SO_4) afforded a pale yellow syrup(0.15g.) which was hydrolysed with sulphuric acid(4%;15c.c.) and gave a reducing syrup. This after treatment with charcoal and filter-cel showed, on a paper chromatogram (with

butanol-ethanol-water, 4:1:5, as the mobile phase) two spots: 1. Rg 0.40, identical with that produced by authentic L-rhamnose; and 2. RG 0.50, identical with that produced by suspected 6-deoxy-L-idose obtained from other sources.

(b) Barium Hydroxide.

Crystalline methyl 3:4-anhydro-6-deoxy-α-L-taloside(0.035g.) was treated with barium hydroxide (1g.) in water(5c.c.) at 100° for 2 hours. Alcohol (7c.c.) was added to the cooled solution and the mixture filtered. The precipitate was washed several times with hot alcohol and the filtrate and washings treated with carbon dioxide. After removal of the solvent the residue was extracted with acetone. The acetone extracts gave a syrup(0.0175g.) $[\alpha]_D^{14°}$-60° (c, 0.4 in water). (cf. methyl α-L-rhamnoside, $[\alpha]_D^{16°}$-62° in water). Hydrolysis with N-sulphuric acid for 4 hours at a 100° gave a reducing syrup, $[\alpha]_D^{16°}$±0°(c, 1.0 in water) which on chromatographic analysis (butanol:ethanol:water,4:1:5, as eluant) showed a strong spot Rg 0.40 identical with one given by authentic L-rhamnose, and a very faint spot Rg 0.50 thought to be 6-deoxyidose.

On standing this syrup partially crystallised. The crystals had mp. 90°

Repetition of this hydrolysis on the residual syrup(X) after removal of the crystals of methyl 3:4-anhydro-6-deoxy-α-L-taloside gave identical results.

Alkaline Hydrolysis of Methyl 3:4-Anhydro-6-deoxy- ∝-L-taloside with Sodium Methoxide.

Methyl 3:4-anhydro-6-deoxy- ∝-L-taloside(2.50g.) was dissolved in dry methanol(150c.c.) containing metallic sodium(2.0g.) and the solution was heated under reflux at 80° for 19 hours. Carbon dioxide was then bubbled through the solution for 6 hours. The precipitated solids were removed by filtration and combined with the residue obtained on taking the filtrate to dryness. The combined solids were extracted with chloroform in the cold(6 x 50c.c.) and removal of the solvent from the combined extracts gave a brown mobile syrup which distilled at 120-130° (bath temperature)/0.1mm. as a colourless syrup(A) (2.0g.), $n_D^{12°}$ 1.4685, $[\alpha]_D^{16°}$ -117° (c, 2.0 in water).

In subsequent preparations of this compound, the passage of gaseous carbon dioxide through the sodium methoxide solution was replaced by the addition of pieces of solid carbon dioxide ("cardice"), the precipitation of sodium carbonate being thereby accelerated.

Removal of the Glycosidic Methoxyl Group.

The syrup(A) (1.2g.) was hydrolysed with N-sulphuric acid solution(52c.c.) until the rotation became constant,($[\alpha]_D^{15°}$ -4°;2 hours). Neutralisation with barium carbonate and removal of the solvent gave a colourless syrup(0.95g.). Chromatographic examination of this syrup with n-butanol:ethanol:water(4:1:5)

as the mobile phase revealed two spots, R_G 0.66 and R_G 0.75, together with traces of free rhamnose, R_G 0.40.

Separation of Hydrolysed Syrup (A) into its Constituent Sugars.

The above mixture (0.90g.) was separated into its components by passage through a column of powdered cellulose(108). The solvent employed for elution was purified light petroleum(b.p.100-120°)-n-butanol (1:1;v/v) saturated with water. The eluate from the column was fractionated into approximately 5cc. portions by the automatic device which changed the receiver after 48-minute intervals. Every fifth tube was analysed chromatographically; fractionation occurred as follows:

Tubes	1-58	-
"	68-88	Fraction I; R_G 0.75
"	89-107	-
"	108-128	Fraction II; R_G 0.66
"	129-149	-
"	150-200	Fraction III; R_G 0.40

After the removal of solvent each of these fractions (as well as syrups obtained from cellulose columns in subsequent separations) were freed from traces of waxy material by dissolution in water followed by warming with decolourising charcoal and filtration through "Hyflo" filtercel. Removal of the water gave in every experiment a colourless syrup which was dried by dissolution in absolute ethanol and removal of the solvent.

Characterisation of the Fractions.

Fraction I. A colourless syrup (0.412g., 45.8%), R_G 0.75, M_G 0.80 (see p. 56), $[\alpha]_D^{16°}$ -14° (c, 0.7 in water), -13° (c, 1.0 in ethanol), $\eta_D^{18°}$ 1.4790.

Found: C, 47.8; H, 8.6; OMe, 18.0. $C_7H_{14}O_5$ requires C, 47.1; H, 7.9; OMe, 17.4%.

This syrup is presumed to be 6-deoxy-3-methyl-L-idose since a syrup with identical R_G value and optical rotation, and identical behaviour on paper ionophoresis (described on p. 54) was obtained from methyl 2:3-anhydro-α-L-taloside (p. 87).

Conversion to the methylglycoside with methanolic hydrogen chloride gave a colourless syrup, $[\alpha]_D^{18°}$ -21°, (c, 2.4 in ethanol).

Anilide Formation.

This fraction (65mg.) dissolved in 5c.c. of an alcoholic solution of aniline [prepared by dissolving freshly distilled aniline (0.55c.c.) in absolute alcohol (10cc.)] containing drierite (0.2g.) was refluxed at 80° for six hours. Removal of the solvent at room temperature afforded a syrup which crystallised completely, m.p. 62-63°, undepressed on admixture with 6-deoxy-3-methyl-N-phenyl-L-idosylamine, m.p. 62-63°, obtained from the 2:3-anhydroderivative (p. 88)

Found: C, 61.0; H, 8.0; N, 4.5. Calc. for $C_{13}H_{19}O_4N$: C, 61.4; H, 7.9; N, 5.4%.

Isolation of a Crystalline Phenylosazone.

This fraction (30mg.) in water (2cc.) was treated with phenylhydrazine (0.15cc.) and glacial acetic acid (2 drops) at 100° for two hours in an atmosphere of carbon dioxide. On cooling in the presence of solid carbon dioxide a yellow solid was deposited. The solid was washed with dilute aqueous acetic acid and allowed to stand in water with solid carbon dioxide at 0° for 20 hours. Filtration, followed by recrystallisation from aqueous ethanol gave light yellow needles, m.p. 122-3°, $[\alpha]_D^{16°}$ 60°(c, 1.6 in ethanol)

Found: OMe, 8.4 ; N, 14.9 ; $C_{19}H_{23}O_3N_4$ requires OMe, 8.7 ; N, 15.8% ;

Fraction II. A colourless syrup (0.302g., 33.5%), RG 0.65, which crystallised completely, m.p. 120°, M_G 0.58 $[\alpha]_D^{15°}$ 15° (c, 1.0 in methanol) (cf. Levene and Muskat (109) who record m.p. 120°, $[\alpha]_D^{20°}$ +13° in methanol for 4-methyl-L-rhamnose); $[\alpha]_D^{17°}$ 14° initial → ±0° (15 mins.) (c, 0.56 in water). Removal of the water from the rotation solution followed by dissolution in ethanol and evaporation of this solvent gave a syrup which crystallised completely on standing, m.p. 120°.

Found: C, 47.2; H, 8.1; OMe, 17.2. Calc. for $C_7H_{14}O_5$ C, 47.2; H, 7.9; OMe, 17.4

Syrupy 4-methyl-L-rhamnose, which had been prepared by methylation of methyl 2:3-isopropylidene-α-L-rhamnoside, followed by removal of the isopropylidene residue and of the glycosidic methoxyl group, when nucleated with a crystal of the above Fraction II

crystallised completely.

Fraction III. A colourless syrup (0.058g., 6.5%), R_G 0.40, crystallised completely, m.p. 92-94° alone or on admixture with authentic L-rhamnose.

Recovery from the column: 86% (after purification of fractions).

A subsequent preparation gave 1.2g. syrup (A) which, after acid hydrolysis(1.02g.) and separation on a cellulose column, gave: Fraction I (0.575g., 56.9%); Fraction II (0.270g., 26.8%); and Fraction III (0.060g., 5.9%), as in the previous preparation, along with a fourth fraction (0.051g., 5.05%) of R_G 0.50, $[\alpha]_D^{16°}$ -27.4°(c, 1.5 in water). Meyer and Reichstein(105) record $[\alpha]_D^{15°}$ -26°(c, 4.462 in water) for 6-deoxy-L-idose.

Recovery from the column: 95%.

Methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside.

Crystalline 3:4-anhydro-6-deoxy-α-L-taloside (3.0g.) was dissolved in neutral methyl iodide(130g.), the solution refluxed at 43° on a water-bath, dry freshly prepared silver oxide(8.0g.) being added in half-gram portions at regular intervals with frequent shaking over 9 hours. The heating was continued for one hour after the last addition of silver oxide; the solution was then cooled and filtered, the silver oxide being extracted with hot chloroform(4 x 30c.c.). The filtrate and extracts were combined and taken to dryness yielding a syrup which was subjected to a

further three treatments as above, its mobility increasing with each methylation. The product from the fourth methylation distilled at 80-100°(bath temperature)/0.01mm. as a colourless, mobile syrup (2.8g.), $n_D^{15°}$ 1.4500, $[\alpha]_D^{16°}$ -140° (c, 0.6 in methanol) (Found: OMe, 35.8; $C_8H_{14}O_4$ requires OMe 35.6%).

Alkaline Hydrolysis of Methyl 3:4-Anhydro-6-deoxy-2-methyl-α-L-taloside with Sodium Methoxide followed by Removal of the Methoxyl Group.

Methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside (3.80g.) was dissolved in dry methanol (250c.c.) containing metallic sodium(3.20g.) and the solution heated under reflux at 90° for 19 hours. The product was worked up as described on p.37, and a mobile syrup (3.73g.), $[\alpha]_D^{16°}$ -60° (c, 1.0 in water) was obtained. This syrup was hydrolysed with aqueous sulphuric acid (4%; 30c.c.) at 100° until the rotation became constant ($[\alpha]_D^{16°}$ -12°; 3 hours). The solution was neutralised with barium carbonate, and, after filtration, the barium salts were extracted with hot chloroform (4 x 15c.c.). The combined extracts and filtrate were taken to dryness and gave a colourless syrup (3.20g.) which partly crystallised on storage at 0°. Yield of crystals: 1.17g.; m.p. 82° - if allowed to recrystallise on the glass slide, re-melted at 78°, $[\alpha]_D^{16°}$ -19° (c, 1.0 in water), +14.5 ⟶ -3° (48 hours, constant) (c, 1.8 in ethanol). Removal of the ethanol from the rotation solution gave a syrup which

crystallised spontaneously, m.p. 78°.

Found: C, 49.5; H, 8.1; OMe, 31.7, $C_8H_{16}O_5$ requires C, 49.9; H, 8.4; OMe, 32.3%.

On the paper chromatogram, using solvent (I) as the mobile phase, the crystals produced a single spot, R_G 0.86. Ionophoresis (p.56-7) gave M_G 0.04 and 0.25.* Chromatographic comparison of the crystals with a syrup, supplied by Dr. P.W. Kent, thought to be 2:4-dimethylrhamnose obtained from natural sources, showed these substances to be chromatographically identical: on chromatographic analysis, identical spots, R_G 0.86 with solvent (I) as eluant, and R_G 0.94 with solvent (II) as the mobile phase, were obtained. Admixture of the two substances produced a single spot on the paper chromatogram with solvent (I) as eluant.

distillation?

The mother liquors (C) (2.03g.) from the crystals (B) on chromatographic analysis revealed a single spot, R_G 0.86, and a faster one, R_G 1.0, with solvent (I) as the mobile phase. Purification of this syrup (C) was affected by passage through a column of powdered cellulose(108) with n-butanol saturated with water - purified light petroleum (b.p.100-120°) (4:6, v/v) as eluant, fractionation being achieved as described on p. 38 . A colourless syrup (1.61g.) was obtained, $n_D^{15°}$ 1.4548, $[\alpha]_D^{18°}$ -18°(c, 1.5 in water, constant), producing a single spot, R_G 0.86, on the paper chromatogram. This syrup partly crystallised on seeding with the crystals (B), (0.8g.). The residual syrup (C)(0.81g.) had $[\alpha]_D^{16°}$ -15°(c, 2.3 in

*These two values were obtained by the use of different types of apparatus.

water), -13°(c, 2.3 in ethanol).

A very small quantity of a second fraction, R_G 1.0, was obtained as a colourless syrup, $[\alpha]_D^{16°}$ +20°, (c, 0.05 in water).

Repetition of these experiments gave in one instance a syrup in 85% yield, R_G 0.86, which crystallised completely, but in no experiment was more than a trace of the faster-moving constituent obtained.

On one occasion, the distillation of the syrup obtained from the methylation of methyl 3:4-anhydro-6-deoxy-α-L-taloside was omitted. Alkaline fission with sodium methoxide followed by acid hydrolysis and separation on a cellulose column gave, in addition to the crystals (B), the following substances: (1) 2:3:4-trimethyl-L-rhamnose(7.3%), R_G 1.01, indistinguishable chromatographically from authentic 2:3:4-trimethyl-L-rhamnose; $[\alpha]_D^{16°}$ + 22°(c, 1.2 in water) (Purdie and Young (111) record $[\alpha]_D^{18°}$ + 25° for 2:3:4-trimethyl-L-rhamnose). (Found: OMe, 44.2. Calc. for $C_9H_{18}O_5$, OMe 45.1%) (2) 6-Deoxy-3-methyl-L-idose(6.05%) which crystallised spontaneously on removal of the solvent, R_G 0.75, m.p. 113-114°, $[\alpha]_D^{17°}$ +14.4° (c, 2.5 in ethanol), -15° constant (c, 1.0 in water).

Found: C, 46.82; H, 7.95; OMe, 17.2. $C_7H_{14}O_5$ requires C, 47.1; H, 7.9; OMe, 17.4%.

Admixture of these crystals with syrupy 6-deoxy-3-methyl-L-idose (p. 39) gave a single spot, R_G 0.75, on the paper chromatogram with solvent (I) as the mobile phase.

Characterisation of the crystalline dimethyl sugar (B)

Anilide formation.

Crystalline dimethyl pentose (B) (0.1g.) dissolved in 6cc. of a solution of re-distilled aniline (0.55cc.) in absolute ethanol (10cc.) containing drierite (0.2g.) was refluxed at 80° for 6 hours. Removal of the solvent at room temperature afforded a syrup which crystallised completely, m.p. 141-142°, $[\alpha]_D^{16°}$ +110° → +7° (20hrs.) (c, 0.4 in ethanol)

Found: C, 60.8; H, 7.5; N, 5.4; OMe, 23.3. $C_{14}H_{21}O_4N$ requires C, 62.9; H, 7.9; N, 5.2; OMe, 23.2%.

An X-ray powder photograph of this anilide was taken by Dr. C.A. Beevers and compared by him with a similar photograph, supplied by the Birmingham laboratories, of a synthetic 2:4-dimethyl-N-phenyl-rhamnosylamine. The two photographs were pronounced identical.

Demethylation of crystals (B).

Attempted demethylation with hydrobromic acid gave only unchanged material in every case. Demethylation with hydriodic acid was then attempted and proved successful. The crystals (B) (0.05g.) were refluxed with freshly distilled hydriodic acid(2cc.) at 100° for 10 minutes. The solution was then diluted with water(15cc.) and neutralised with silver carbonate. Removal of silver ions was effected by passage of hydrogen sulphide through the solution; after filtration and removal of excess hydrogen

sulphide by aeration, the filtrate was shaken with small portions of resins (Amberlite IR-100-H and IR-4B-OH) for complete clarification. It was then taken to dryness affording a colourless syrup which, on heavy spotting, produced on the paper chromatogram a single spot, R_G 0.40, identical with that given by authentic L-rhamnose.

Oxidation of the crystalline dimethyl sugar(B) with bromine to the corresponding lactone.

The crystals (B) (0.1g.), strongly reducing towards Fehling's solution, were dissolved in water (2c.c.) and bromine (3c.c.) was added. The solution was kept at 15° for 96 hours, after which a portion of it had, when freed from bromine by aeration, no action on boiling Fehling's solution. The bromine was removed by aeration and the solution neutralised with silver carbonate and filtered. The filtrate was successively shaken with cation and anion exchange resins (Amberlite IR-100-H and IR-4B-OH) and, after removal of the resins by filtration, taken to dryness to give an acid which distilled at 135°(bath temperature)/0.01mm. as a colourless syrup(75mg.), $[\alpha]_D^{16°}$ + 47° (15mins.) (c, 0.9 in water) unchanged after 70 hours. Attempts to form a crystalline amide were unsuccessful.

Attempted Phenylhydrazide Formation.

Dimethylrhamnonolactone(30mg.) was dissolved in an alcoholic solution of phenylhydrazine(0.7g. phenyl-

hydrazine in 20c.c. ethanol) and the solution was heated at 85° for two hours under reflux. It was then further heated on the water-bath at 80° until a syrup remained; this appeared to crystallise on storage at 0° and trituration with ether, but it was not possible to obtain a melting point of the crystals.

Formation of Glycoside of the Dimethyl Sugar.

The crystalline dimethyl sugar (B) (0.155g.) was treated with methanolic hydrogen chloride (10c.c.; 1%) until non-reducing towards Fehling's solution(4 hours). After neutralisation with silver carbonate and removal of the solvent a colourless syrup was obtained (0.150g.)

Methylation of the Dimethyl Glycoside.

The above syrup (0.150g.) was subjected to two methylations with silver oxide and methyl iodide and the product distilled at 80-100°(bath temperature)/ 0.05mm. A colourless, mobile syrup (D) (0.145g.) was obtained, $\eta_D^{16°}$ 1.4421, $[\alpha]_D^{16°}$ -17° (c, 1.5 in water). Hirst and Macbeth(112) record for methyl 2:3:4-trimethyl-L-rhamnoside (mixture of α- and β- forms) $\eta_D^{16°}$ 1.4423, $[\alpha]_D^{16°}$ -15° in water.

(Found: OMe, 55.6; calc. for $C_{10}H_{20}O_4$, OMe, 56.3%). On chromatographic analysis, with solvent (I) as the eluant and using a phosphoric acid-aniline oxalate spray, this syrup gave a single spot very slightly faster than that produced by tetramethyl-D-glucose, obviously corresponding to a fully methylated sugar.

A mixture of authentic methyl 2:3:4-trimethyl-L-rhamnoside and a portion of this syrup produced on the paper chromatogram, with the above eluant and spray, a single spot, R_G 1.01.

Hydrolysis of Syrup (D) to the Trimethyl sugar.

Syrup (D) (0.1g.) was hydrolysed with aqueous sulphuric acid (N.,10c.c.) at 100° until the rotation was constant, ($[\alpha]_D^{16°}$ -17° → -12°, 2 hours). (Authentic 2:3:4-trimethyl-L-rhamnose has $[\alpha]_D^{16°}$ +27°). Neutralisation with barium carbonate followed by filtration and removal of the solvent gave a colourless syrup(E), $[\alpha]_D^{16°}$ -24° (c, 0.8 in ethanol). (Hirst and Macbeth, loc. cit., record $[\alpha]_D^{16°}$ -9° in ethanol for trimethyl-L-rhamnose).

Formation of the Anilide.

The above syrup (80mg.) was treated with aniline (0.18cc., in alcohol, 30c.c.) at 80° for 7 hours, after which the mixture was allowed to evaporate in a desiccator at 15°. Crystals were deposited after some days. Recrystallisation from light petroleum-ether (b.p.40-60°)-alcohol gave colourless crystals in very poor yield, m.p. 111-112° undepressed on admixture with 2:3:4-trimethyl-L-rhamnose anilide (m.p. 111-112°).

Oxidation with Nitric acid of the Fully Methylated Sugar (D).

Syrup (D) (0.30g.) was dissolved in nitric acid

(5.5c.c.; density 1.4) and the solution gently heated to 50° over two hours; the temperature was then slowly raised to 90° (112). After 2.5 hours at this temperature, water (5c.c.) was added and the heating at 90° continued for a further three hours. At the end of this period, water was added and the solution concentrated under diminished pressure almost to dryness. This process was repeated until, after several days, all the nitric acid had been removed as shown by testing the distillate for NO_3^- [the test involves reduction of NO_3^- to NO_2^- with zinc dust and acetic acid; sulphanilic acid is then diazotised with the NO_2^- forming a diazonium salt which reacts with -naphthylamine to give a red dyestuff. A red to pink colour obtained on testing the distillate with zinc-acetic acid and sulphanilic acid-α-naphthylamine then indicates NO_3^- (118)]. The last traces of water were finally removed by solution in methanol and removal of the solvents gave a colourless syrup.

This syrup (0.06g.) was heated at 70° with a solution of methanolic hydrogen chloride (3.5c.c.; 2%; 6 hours). After neutralisation and removal of the solvent, a colourless syrup (0.055g.) was obtained, $[\alpha]_D^{16°}$ +26.6° (c, 0.6 in water) (cf. Hirst and Macbeth (112) who record $[\alpha]_D^{16°}$ +47° in methanol for L-<u>arabo</u>trimethoxyglutaric acid dimethyl ester).

The above dimethyl ester (0.055g.) was dissolved in methanolic ammonia and the solution kept at 15° for

72 hours. Removal of the solvent and storage of the syrup at 0° brought about partial crystallisation. The crystals had (after tiling, separation and recrystallisation from ethyl acetate) m.p. 230°, alone and on admixture with an authentic specimen. This product had $[\alpha]_D^{16°}$ +52°(c, 0.5 in ethanol) (cf. Hirst and Macbeth, who record $[\alpha]_D^{16°}$ +50.4° in water and m.p. 230° for L-arabotrimethoxyglutardiamide).

It would thus appear that the product of the nitric acid oxidation of the fully methylated sugar(D) obtained from the crystalline dimethyl sugar(B) is L-arabotrimethoxyglutaric acid. This would indicate that syrup (D) is methyl 2:3:4-trimethyl-L-rhamnoside and the crystals (B) are 2:4-dimethyl-L-rhamnose.

Characterisation of the Residual Syrup (C_1) after the removal of crystals (B.)

This syrup was chromatographically identical with the crystals (B) and ionophoresis of the two substances failed to show any difference between them.

Demethylation of the syrup (0.2g.) as for the crystals gave a colourless syrup, $[\alpha]_D^{16°}$ +4°(c, 2.0 in water) (cf. L-Rhamnose, $[\alpha]_D^{16°}$ +8° in water) which on a paper chromatogram produced a single spot, R_G 0.40, identical with L-Rhamnose.

Formation of the Anilide.

A portion of syrup (C_1) (80mg.) was converted into the anilide according to the method described under

the dimethylpentose (B); crystals were obtained which, after recrystallisation from ethyl acetate containing a little light petroleum (b.p.40-60°) had m.p. 141-2° $[\alpha]_D^{16°}$ +100° ⟶ +7° (20hrs.) (c, 0.5 in ethanol)

Conversion to the Fully Methylated Sugar.

A portion of syrup (C_1) (0.140g.) was converted to the glycoside by treatment with methanolic hydrogen chloride (1.4%) for 4 hours at 75°. A colourless syrup was obtained which was subjected to two methylations with methyl iodide and silver oxide. Distillation of the resulting syrup at 100° (bath temperature)/ 0.1mm. gave a non-reducing syrup (0.0525g.), $n_D^{16°}$ 1.4421, $[\alpha]_D^{16°}$ -16° (c, 0.52 in water) cf. methyl 2:3:4-trimethyl-L-rhamnoside, $n_D^{16°}$ 1.4423, $[\alpha]_D^{16°}$ -15° in water (α - β mixture) . Hydrolysis to the free sugar with N. sulphuric acid was complete in 3½ hours. A colourless syrup, $[\alpha]_D^{16°}$ +5° (c, 1.0 in water) was obtained, R_C 1.01 with solvent (I) as eluant. This was identical with the spots given by synthetic 2:3:4-trimethyl-L-rhamnose. Attempts to isolate a crystalline anilide were unsuccessful.

Synthesis of Authentic Trimethyl-L-Rhamnose.

L-Rhamnose-H_2O (1.0g.), after dehydration with benzene and ethanol (6 x 20c.c.)(1:1,v/v), was converted into the methyl α-L-rhamnoside (1.0g.) by treatment with methanolic hydrogen chloride (2%;5c.c.) at

80° for 40 hours. The rhamnoside was then methylated by 4 treatments with methyl iodide and silver oxide and methyl 2:3:4-trimethyl-L-rhamnoside (0.92g.) was obtained; it had $\eta_D^{16°}$ 1.4420.

(Found: OMe, 55.3; calc. for $C_{10}H_{20}O_5$ OMe, 56.3%). Hydrolysis of the syrup (0.5g.) with aqueous sulphuric acid (4%; 30c.c.) until the rotation became constant $\{[\alpha]_D^{16°}+30°$(c, 2.0 in water; 3 hours)$\}$ gave a colourless syrup. Purdie and Young (111) record $[\alpha]_D^{16°}+27°$ and Hirst and Macbeth (112) $[\alpha]_D^{16°}+25°$ in water for 2:3:4-trimethyl-L-rhamnose. Chromatographic analysis of this syrup and the syrup E ($[\alpha]_D^{16°}$ -24°) showed a single spot R_G 1.01 with solvent (I) as the eluant.

2:5-Dichlorophenylhydrazine Derivatives of:-

(a) Rhamnose. L-Rhamnose-H_2O(0.5g.) was added to 2:5-dichlorophenylhydrazine (0.5g.) in hot methanol (5c.c.). The clear solution was evaporated to a syrup on the boiling water-bath, crystals appearing in the hot solution. A little ether was added to prevent the formation of a solid mass. After cooling, the crystals were washed with alcohol and then with ether, m.p. 170-171°(cf. Neuberg and Mandl(119) who record m.p. 171° for L-rhamnose 2:5-dichlorophenylhydrazone).

(b) Synthetic 2:3:4-trimethyl-L-rhamnose (0.3g.) was treated with 2:5-dichlorophenylhydrazine as above, but only unchanged material was recovered.

(c) Syrup E, on similar treatment, was recovered unchanged.

Attempted 2:4-dinitrophenylhydrazone Formation.

2:3:4-Trimethyl-L-rhamnose(0.1g.) in water(1 cc.) was treated with glacial acetic acid(1c.c.) and 2:4-dinitrophenylhadrazine(0.08g.). The mixture was heated at 50° for 20 minutes and then stored at 15° for 18 hours. Unreacted materials were the only products (2:4-dinitrophenylhydrazine, m.p. 198°).

This experiment was repeated using L-rhamnose-H_2O(1g.) and in this case L-rhamnono-2:4-dinitrophenylhydrazone(0.9g.) was obtained, m.p. 163-164° (4 recrystallisations from alcohol-ether). (cf.Domínguez (120) who records m.p. 164° for this derivative).

2:3-Dimethyl-L-Rhamnose(107).

Methyl 4-toluene-p-sulphonyl-α-L-rhamnoside (1.25g.) was subjected to three methylations with methyl iodide and silver oxide; the product(1.20g.), isolated in the usual way, formed large crystals which, after recrystallisation from warm methanol, had m.p. 110-111°, $[\alpha]_D^{16°}$ -32° (c, 3.0 in chloroform).

Found: C, 52.8; H, 6.4; OMe, 25.6. Calc. for $C_{16}H_{24}O_7S$ C, 53.3; H, 6.7; OMe, 25.8%.

Methyl 2:3-dimethyl-4-toluene-p-sulphonyl α-L-rhamnoside(1.20g.) in methanol(30c.c.) and water(10c.c.) was treated with sodium amalgam(10g.;4%) at 35°, with stirring, for 10 hours. After filtration and extraction with chloroform, the extracts were treated with carbon dioxide for 30 minutes, dried over anhydrous

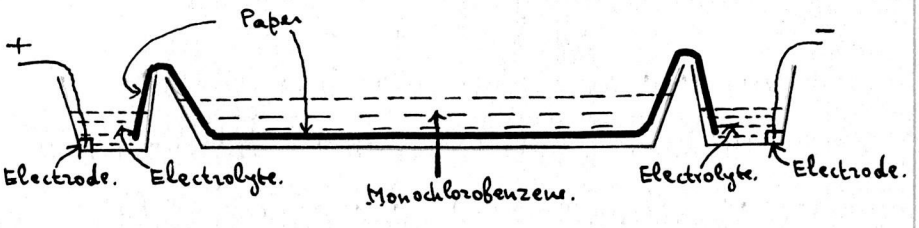
sodium sulphate and freed from organic solvents. The aqueous residue was neutralised with carbon dioxide, evaporated to dryness and extracted with ether (4 x 15c.c.). From these operations, methyl 2:3-dimethyl-α-L-rhamnoside(0.50g.) was obtained as a syrup, 1.4540, $[\alpha]_D^{16^\circ}$ -5° (c, 1.5 in water). This syrup was hydrolysed with aqueous sulphuric acid (4%;25c.c.) at 100° until the rotation became constant ($[\alpha]_D^{16^\circ}$ + 38°; 6 hours).

(Found: OMe, 31.1; $C_8H_{16}O_5$ requires OMe, 32.3%).

Treatment of the 2:3-dimethyl-L-rhamnose(0.3g.) in ethanol(2.5c.c.) with freshly distilled aniline (0.3g.) at 80° for two hours, followed by evaporation at room temperature, gave a syrup which, on seeding with a crystal of 2:3-dimethyl-L-rhamnose anilide, crystallised and had m.p. 138-140° alone or on admixture with authentic 2:3-dimethyl-L-rhamnose anilide.

Experiments with Filter Paper Ionophoresis

(a) Following the method of Consden and Stanier(121)



Whatman No.1 paper (50cm. long, spotted with the sugars under examination at a distance of 14cm. from the top) was dipped into a buffer solution (borax solution consisting of 12.403g. boric acid + 100c.c. N.

sodium hydroxide solution/litre-0.1 N. sodium hydroxide solution; 6:4, v/v, so as to produce a pH of 10), blotted to remove excess buffer solution and fixed onto a rectangular glass frame designed to maintain the paper stretched horizontally. The ends of the paper dipped into plastic troughs disposed on either end of a porcelain dish into which the glass frame and paper were placed. The plastic troughs were filled with the electrolyte (buffer solution) and the dish with monochlorobenzene (chosen because of its density which is approximately that of wet paper). Care was exercised to maintain the equalised levels of electrolyte in the troughs below the level of the chlorobenzene in the dish. After allowing 15 minutes for equilibration, a potential of 400 volts was applied from a rectifier for 4 hours. At the end of this period, the paper was removed and dried at 15°; the sugars were revealed by spraying with aniline oxalate-glacial acetic acid (5:1,v/v). After a few minutes heating at 100-110°, the paper was viewed in u.v. light, in which some of the monose spots have an intense fluorescence.

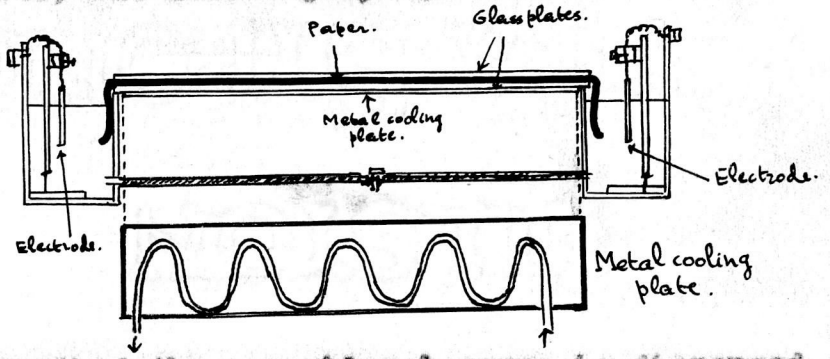
Filter paper ionophoresis under these conditions has been used in borate buffer at pH 10 by Foster and Stacey(122) to study the migration of simple carbohydrate derivatives. As an index of ionophoretic movement the arbitrary term M_G has been suggested(114) where,

$$M_G = \frac{\text{True distance of migration of a substance}}{\text{True distance of migration of D-glucose}}$$

"true" distances of migration being those that have been corrected for movement due to electroendosmotic flow by reference to the non-complex-forming 2:3:4:6-tetramethyl-D-glucose. The following M_G values have been determined:

Substance		M_G
6-deoxy-3-methyl-L-idose	(pp. 39,87)	0.80
2-methyl-L-fucose	(p. 88)	0.33
6-deoxy-L-idose	(p. 88)	1.0
2:3-dimethyl-L-rhamnose	(p. 54)	0.02
2:4-dimethyl-L-rhamnose	(p. 43)	0.04
~~3:4-dimethyl-L-rhamnose~~	~~(p.~~	
4-methyl-L-rhamnose	(p.40)	0.58
L-fucose	(p.88)	0.92

(b) An apparatus similar to that described by Foster(123) was also employed.



By this method the monochlorobenzene is dispensed with; the Whatman No.1 paper (68cm. long) was spotted with the sugars, the origin line being drawn half-way between the ends. The paper was then immersed in buffer of pH10 almost up to the origin line and then blotted to remove excess buffer; the second half of the strip was treated likewise, care being taken to achieve two liquid fronts near to and equidistant from the origin line. The moist strip

was then inserted between two glass plates and these were clamped on to a metal cooling plate (consisting of a sheet of tinned iron on to which a copper tube was soldered in a meandering pattern, a flow of cold water through this tube affording efficient cooling). With this apparatus relatively high voltages (750-1000 volts) were used. The ends of the paper strip dipped into the buffer solution as in the previous arrangement; on termination of the ionophoresis the paper was dried and developed as before.

By means of this high voltage ionophoresis no difference could be detected between 2:3- and 2:4-dimethyl-L-rhamnose. They were both found to have M_G 0.25; no distinction was afforded between crystalline and syrupy 2:4-dimethyl-L-rhamnose which both had M_G 0.25 (potential of 1000 volts). The following M_G values have been determined using this high-voltage method:

Substance	M_G
2:3-dimethyl-L-rhamnose	0.25
2:4-dimethyl-L-rhamnose	0.25
3:4-dimethyl-L-rhamnose	0.34

DISCUSSION.

Crystalline anhydrous rhamnose (m.p.122-126°) was obtained by Fisher(110) by repeated heating of the monohydrate on a water-bath and crystallisation from acetone; this product was almost entirely the β-form of the sugar. Purdie and Young(111) attempted the dehydration by heating rhamnose-H_2O for long periods under the conditions used to dry a sample before analysis; they found dehydration by these means to be incomplete, a rise in m.p. from 86-88° to 105° showing that the change $\alpha \longrightarrow \beta$ had occurred to a considerable extent. These authors were more successful when they brought rhamnose-H_2O into solution by prolonged boiling with 30 times its weight of dry acetone containing 9 parts of ethanol: on cooling, β-rhamnose, m.p. 122-124, was obtained. Efficient dehydration of the monohydrate was achieved in this work by bringing it into solution by heating with a mixture of ethanol and benzene(1:1); six treatments afforded the anhydrous sugar, m.p. 120°.

Methyl α-L-rhamnoside was obtained crystalline by two methods. Heating at reflux temperature with 0.25% methanolic hydrogen chloride solution until non-reducing gave the glycoside in good yield (90-95%) but the reaction required an average of 40 hours for completion; also, it was necessary repeatedly to extract the silver salts resulting from neutralisation

of the acid solution with silver carbonate and contam-:ination with Ag^+ could not always be avoided. An alternative procedure employed involved the heating at reflux temperature of anhydrous rhamnose in dry methanolic solution in the presence of an equal weight of cation exchanger (Amberlite Resin IR-100-H). By this method the time necessary for the methanolic solution to become non-reducing was reduced to 6-8 hours and neutralisation with the attendant Ag^+ cont-amination was avoided. On the other hand, yields (75-80%) were not as good as with the former method in spite of exhaustive washing of the resin. Also, for larger quantities of sugar, correspondingly large amounts of cation exchanger were required, when acid-ity developed rendering neutralisation again necessary at the end of the operation. As a rule, therefore, the resin method was reserved for the formation of glycosides of relatively small quantities (not exceed-:ing 3g.) of sugars, the methanolic hydrogen chloride procedure being employed in other instances.

The condensation of the rhamnoside with acetone took place with the two cis-situated hydroxyl groups at carbon atoms 2 and 3, and methyl 2:3-isopropylidene-α-L-rhamnoside was obtained. For effective conden-sation it was necessary to employ freshly dehydrated and finely divided copper sulphate, vigorous shaking for 120 hours being desirable.

Treatment of the above compound with toluene-p-

sulphonyl chloride could only have resulted in substit-:ution in position C_4 and, as the reaction was carried out in pyridine, the hydrogen chloride liberated during this substitution was consumed as the stable pyridine hydrochloride. As the methyl 2:3-*iso*propylidene-4-toluene-*p*-sulphonyl-α-L-rhamnoside was crystalline, maximum purity of this derivative could be obtained. Methanolic hydrogen chloride(1%) was found to be the best reagent for removal of the *iso*propylidene group and methyl 4-toluene-*p*-sulphonyl-α-L-rhamnoside was obtained as an acetone-free, non-feducing syrup.

The removal of the toluene-*p*-sulphonyl group by the addition of the exact quantity of sodium hydroxide was achieved by the use of phenolphthalein indicator; crystalline sodium toluene-*p*-sulphonate was deposited and the resultant anhydro derivative was extracted from the crystalline deposit with ethyl acetate.

Earlier work(23) on the removal of toluene-*p*-sulphonyl groups from sugars has proved that this will take place with ease if there is an adjacent free hydroxyl group in the *trans*- position relative to the toluene-*p*-sulphonyl group, with the formation of an ethylene oxide-ring between the carbon atom which carried the *trans*- situated hydroxyl group and the carbon atom carrying the toluene-*p*-sulphonyl group, Walden inversion taking place on the latter. Methyl

4-toluene-p-sulphonyl α-L-rhamnoside has a trans-situated free hydroxyl group on carbon atom 3 and removal of the OTs^- anion caused inversion on carbon atom 4 and the formation of an ethylene oxide ring between C_3 and C_4. Removal of the solvent from the ethyl acetate extracts followed by recrystallisation from light petroleum (b.p.40-60°) gave methyl 3:4-anhydro-6-deoxy- α-L-taloside as a pure crystalline substance.

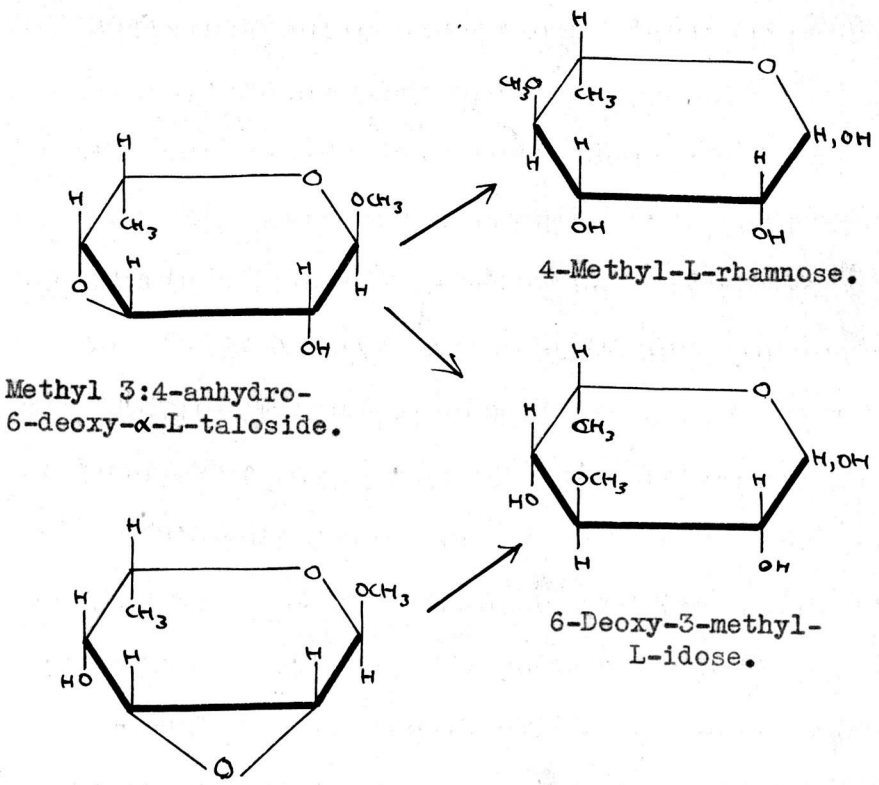
Fission of the ethylene oxide ring can take place in two ways and, depending on which oxygen bridge breaks, a 6-deoxymannoside (rhamnoside) or a 6-deoxyidoside derivative is obtained. Hydrolysis with potassium hydroxide followed by acid hydrolysis of the glycosidic group gave a syrup which showed on the paper chromatogram both 6-deoxy-L-mannose (L-rhamnose) and 6-deoxy-L-idose. Hydrolysis with barium hydroxide followed by acid hydrolysis gave chiefly 6-deoxy-L-mannose with only a trace of 6-deoxy-L-idose, as did also direct acid hydrolysis of the anhydro compound. Crystalline 6-deoxy-L-mannose was in fact isolated from the last two hydrolyses. This agrees with the result of Müller(42) who obtained both D-glucose and D-gulose by the action of acid on methyl-3:4-anhydro- β-D-galactoside.

When sodium methoxide is used to cleave the epoxide ring, then, in all the examples previously studied, fission is followed by the attachment of the

-OMe radical to one of the carbon atoms, Walden inversion occurring at the point of entry of this group. With sodium methoxide on methyl 3:4-anhydro-6-deoxy- α -L-talosside, if the carbon-oxygen bond nearer the glycosidic methoxyl breaks, then methyl 6-deoxy-3-methyl-L-idoside would be obtained, while fission at the bond further from the glycosidic methoxyl would give rise to methyl 6-deoxy-4-methyl-L-mannoside (methyl 4-methyl-L-rhamnoside). Experimentally both these derivatives were isolated as the free sugars after acid hydrolysis, the former in 45.8% yield and the latter in 33.5% yield. In subsequent preparations, by altering the conditions slightly, the proportions of these two derivatives varied, but in every experiment the 6-deoxy-L-idoside was obtained in the larger quantity. Small quantities of the methylglycosides of rhamnose and 6-deoxy-L-idose were also obtained and isolated as the free sugars. Percival and Zobrist(39) also recorded the isolation of small amounts of free xylose and arabinose from the sodium methoxide fission of methyl 2:3-anhydro-D-lyxoside and so far as the author is aware, this is the only published record of complete analysis of the fission products.

After fission with sodium methoxide the product was hydrolysed to the free sugar with aqueous sulphuric acid and the sugars separated by passage through a cellulose column, the resultant syrups being purified

by dissolution in water and treatment with decolourising charcoal and filtered. The 6-deoxy-3-methyl-L-idose was obtained as a colourless syrup, $[\alpha]_D^{16°}$ -14°, which analysed correctly for a 6-deoxy monomethyl hexose and was chromatographically identical with a syrup which also had $[\alpha]_D^{16°}$ -14° and was obtained from the scission products of methyl 2:3-anhydro-6-deoxy-α-L-taloside(p.87). Both syrups were characterised by the isolation of an identical crystalline anilide, 6-deoxy-3-methyl-N-phenyl-L-idosylamine, m.p.60° alone and on admixture. The same crystalline phenylosazone was also prepared from both these syrups. The only derivative which could be obtained directly from the fission of both methyl 3:4-anhydro- and methyl 2:3-anhydro-6-deoxy-L-taloside is methyl 6-deoxy-3-methyl-L-idoside.



A small quantity of crystalline 6-deoxy-3-methyl-L-idose was obtained in one experiment from the sodium methoxide fission of methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside due to incomplete methylation of the anhydro compound. This substance analysed correctly from a 6-deoxy-monomethyl hexose and had the same R_G in solvent (I) and the same rotation in water as the syrupy product; the rotation in ethanol was of the same magnitude but opposite in sign.

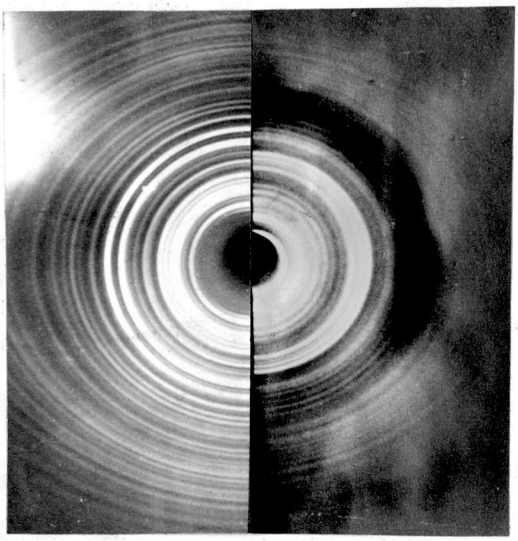
The 6-deoxy-4-methyl-L-mannose (4-methyl-L-rhamnose) was isolated as a syrup which crystallised completely. Its constants agreed well with those recorded for 4-methyl-L-rhamnose and the addition of a crystal of it to syrupy 4-methyl-L-rhamnose, prepared by the methylation of methyl 2:3-isopropylidene α-L-rhamnoside followed by the removal of the isopropylidene and glycosidic groups, caused complete crystallisation to take place.

The isolation of both possible fission products from methyl 3:4-anhydro-6-deoxy-L-taloside is in direct conformity with the results of Peat and Wiggins (24); these authors obtained from methyl 3:4-anhydro-2:6-dimethyl-β-D-alloside on alkaline hydrolysis with sodium methoxide a mixture of methyl 2:3:6-trimethyl-β-D-glucoside and methyl 2:4:6-trimethyl-β-D-guloside in the proportions of 2:1; it does not, however, support the statement of Bose et al.(103) that "the opening of the 3:4-epoxide ring seems to follow the rule that the hydroxyl group at position 4 must be

trans to the bulky primary hydroxyl group". It may well be, however, that the $-CH_3$ group which, in the present experiments, has replaced the primary hydroxyl group, has less influence on the scission of the epoxide ring.

Due to its sensitivity towards changes in pH, methyl 3:4-anhydro-6-deoxy-α-L-taloside was methylated with methyl iodide and silver oxide(Purdie). The extent of the methylation was followed by micro-Zeisel determinations of methoxyl after each methylation. After four methylations the methoxyl content had reached a constant value and methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside was isolated as a colourless mobile syrup.

Fission of the epoxide ring in this 2-methyl derivative with sodium methoxide could give, depending on which oxygen bond breaks, methyl 6-deoxy-2:3-dimethyl-L-idoside or methyl 6-deoxy-2:4-dimethyl-L-mannoside (methyl 2:4-dimethyl-L-rhamnoside). In practice, a single deoxydimethyl hexoside (with traces of contaminating trimethyl sugar) was obtained. After hydrolysis with aqueous sulphuric acid, a colourless syrup was obtained which partly crystallised. After removal of the crystals (B) (37%) the mother liquors were purified by elution through a cellulose column and a further yield of crystals (B) (50% of the mother liquors) was obtained, and in one experiment complete crystallisation took place.



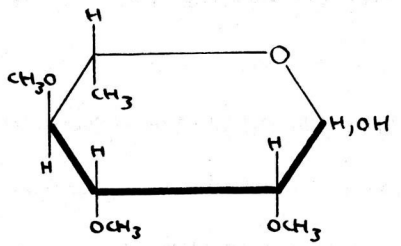
Crystals (B). Dimethylrhamnose supplied.

Comparison of X-ray powder photographs of crystals(B) and of dimethyl rhamnose supplied by Dr. P.W. Kent and pronounced by him to be different.

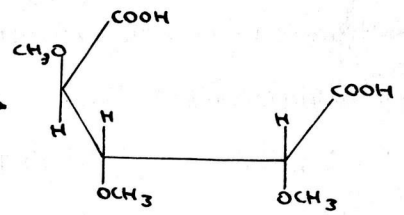
The crystals (B) analysed correctly for a 6-deoxy-dimethyl hexose and on demethylation gave a syrup which on chromatographic analysis revealed only L-rhamnose. The crystals would therefore appear to be 6-deoxy-2:4-dimethyl-L-mannose (2:4-dimethyl-L-rhamnose). However, a private communication (unpublished work) from Dr. P.W. Kent informed the author that he had isolated a crystalline dimethyl-rhamnose from natural sources which he considered to be the 2:4-dimethyl derivative and, although his substance was chromatographically identical with the crystals (B), X-ray powder photographs showed the two substances to be different.

Experiments were carried out to characterise crystals (B): a crystalline anilide was isolated which analysed correctly for a 6-deoxy-dimethyl-N-phenyl hexosyl-amine. Lactone formation gave a syrupy δ-lactone which failed to yield a crystalline amide or phenylhydrazide. Glycoside formation followed by complete methylation gave a syrupy methyl 6-deoxytrimethyl hexoside (D) chromatographically identical with methyl 2:3:4-trimethyl-L-rhamnoside; moreover, the specific rotation of (D) agreed with that reported by Hirst and Macbeth(112) for an α - β mixture of methyl 2:3:4-trimethyl L-rhamnosides. Hydrolysis with acid, however, gave a trimethyl sugar whose final rotation in water was slightly negative, in contrast to the value of $[\alpha]_D^{18°} + 27^0$ recorded for

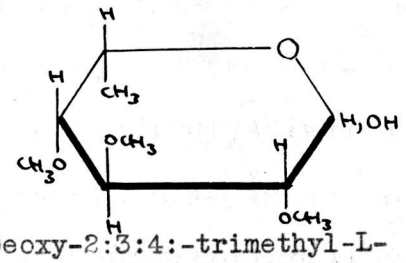
2:3:4-trimethyl-L-rhamnose. Anilide formation gave a poor yield of the crystalline anilide, m.p. 112° alone or on admixture with 2:3:4-trimethyl-N-phenyl-rhamnosylamine. Oxidation of the trimethyl sugar with nitric acid followed by ester and amide formation gave a partly crystalline syrup. The rotation of this syrup was very similar to that recorded for L-arabotrimethoxyglutaric acid diamide, and the crystals of the amide when freed from syrup had m.p. 230° alone and on admixture with authentic L-arabo trimethoxyglutaric diamide. Had the 6-deoxy-dimethylhexoside (B) been the second possible derivative from ring fission, namely methyl 6-deoxy-2:3-dimethyl-L-idose, then glycoside formation and complete methylation followed by nitric acid oxidation would yield inactive xylotrimethoxyglutaric acid.



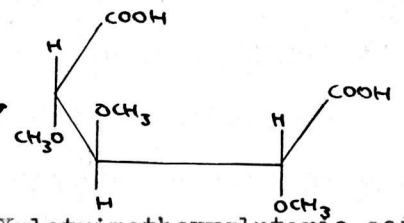
2:3:4-Trimethyl-L-Rhamnose.



L-Arabotrimethoxyglutaric acid.



6-Deoxy-2:3:4:-trimethyl-L-idose.



i-Xylotrimethoxyglutaric acid.

More recently, the Birmingham laboratories have carried out a synthesis of 2:4-dimethyl-L-rhamnose by

the use of trifluoroacetyl derivatives, a brief mention of which is made in a recent journal(113), but no detailed account of this synthesis has appeared so far. Through the courtesy of Professor Stacey the author was supplied with the constants of this synthetic derivative and an X-ray powder photograph of the anilide. Below is a comparison of crystals (B) with the Birmingham 2:4-dimethyl-rhamnose

Properties	2:4-Dimethyl-L-rhamnose synthesised at Birmingham.	6-Deoxy dimethyl hexoside isolated from methyl 3:4-anhydro-6-deoxy-2-methyl-L-taloside.
Physical state	Hygroscopic solid, m.p. 91-92°	Needles, m.p. 82°
Specific rotation	+7° in ethanol.	+14.5° → -3° in ethanol(24 hours)
R_G	0.87	0.86
Anilide	m.p.141-142°	m.p.141-142°
Anilide (ethanol)	+136° → +4° (24 hrs.)	+110° → +7° (20 hrs.)

Through the kindness of Dr. Beevers an X-ray powder photograph of the anilide was taken which proved to be identical with the photograph from Birmingham of their anilide.

In view of all the above results it is therefore concluded that crystals (B) are 6-deoxy-2:4-dimethyl-L-mannose (2:4-dimethyl-L-rhamnose).

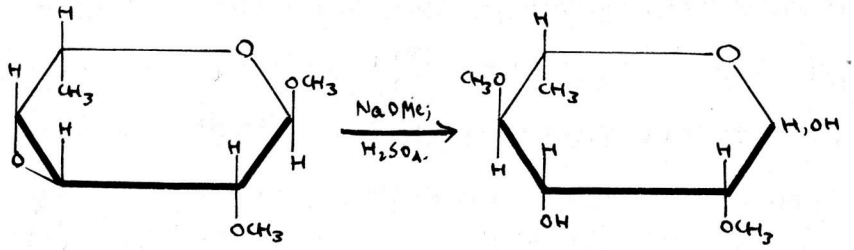
Since the other possible fission product, 6-deoxy-

2:3-dimethyl-L-idose, might be present in the mother liquors syrup (C_1) in spite of the chromatographic identity of this substance with the crystals (B), experiments were carried out to prove the constitution of this syrup. A crystalline anilide was prepared which had m.p.141-142° undepressed on admixture with the anilide from the crystals (B). Demethylation experiments gave only rhamnose and glycoside formation followed by complete methylation afforded a syrup with constants closely resembling those of methyl 2:3:4-trimethyl-L-rhamnoside and indistinguishable from it on chromatographic analysis.

It seems very unlikely that the syrup contains any 6-deoxy-2:4-dimethyl-L-idose since very heavy spotting of the demethylated syrup (C_1) failed to reveal any trace of the other sugar; furthermore, the specific rotation of the demethylated syrup, $[\alpha]_D^{16°}$ +6°, is very close to that recorded for L-rhamnose, $[\alpha]_D^{18°}$ +8°, while 6-deoxy-L-idose has $[\alpha]_D^{7°}$ -26°. In view of the difference in rotation (crystals, $[\alpha]_D^{16°}$ +14.5 → -3° in ethanol; syrup (C_1), $[\alpha]_D^{16°}$ -13° in ethanol) the most tempting hypothesis is that the syrup (C_1) is a mixture of the α-β-forms and the crystals represent the β-form of 2:4-dimethyl-L-rhamnose.

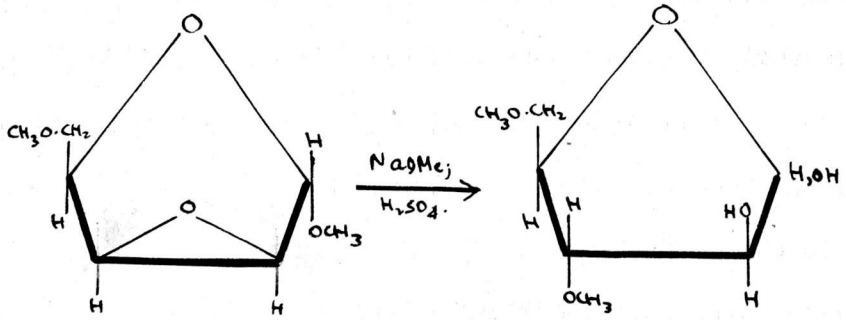
While in the unmethylated derivative fission of both oxygen bridges takes place and the two expected monomethyl derivatives were isolated, the position of

a methoxyl residue in position C_2 appears to cause a preferential splitting of the ring: the oxygen bridge further from the methoxyl group breaks and the entering OMe radical attaches itself to carbon atom 4 in a trans- position relative to the CH_3 group on carbon atom 5. This is in agreement with the results of Percival and Zobrist(39) who obtained only 3:5-dimethyl-D-arabinose from the cleavage with sodium methoxide of methyl 2:3-anhydro-5-methyl-D-lyxoside: ~~here too, the entering OMe radical attaches itself to a carbon atom trans-situated with respect to the OMe group already in the molecule.~~



Methyl 3:4-anhydro-2-methyl-6-deoxy-α-L-taloside.

2:4-Dimethyl-L-rhamnose.



Methyl 2:3-anhydro-5-methyl-D-lyxoside.

3:5-Dimethyl-D-arabinose.

It is perhaps also relevant to point out that in so far as the $-CH_3$ group in rhamnose can be compared with the primary hydroxyl group, this result is in agreement with the contention of Bose et al. (103) that in the fission of 3:4-anhydro rings the hydroxyl at C_4 must be <u>trans</u>-situated with respect to the primary hydroxyl group.

EXPERIMENTAL.

(b) Derivatives from L (-)Fucose.

Of a variety of substances in which L-fucose is present the best source of this sugar is the genus Fucaceae of the common brown seaweeds (Phaeophyceae). L-Fucose was obtained crystalline from the brown seaweed Fucus vesiculosus in two ways:

1. by processing the whole weed, and
2. by first preparing fucoidin and then extracting the sugar from this polysaccharide.

1. Preparation of L-Fucose from Fucus vesiculosus.

The weed, kindly supplied by the Scottish Seaweed Research Association, Inveresk, Midlothian, was collected at Ardbann Bay, Kerrera Sound, in August 1950. After washing with water and removal of the large shells, the seaweed was dried on a rack at 25-30° for 48 hours, and ground in a Christy and Norris mill fitted with a 1/64" mesh screen.

The dried and milled weed (10kg.) was soaked in cold 3% (w/v) aqueous sulphuric acid (3 litres) for 18 hours. After removal of the acid by decantation and filtration, the seaweed was heated with 2% (w/v) aqueous sulphuric acid (8 litres) at 100° for 72 hours. The undissolved material was removed by filtration and thoroughly washed with water, the washings (3 litres) being collected and combined with the filtrate.

The sulphuric acid in the filtrate was neutralised to litmus with barium carbonate (150g.), a few drops of capryl alcohol being added to control foaming. The barium salts were removed by filtration and the clear filtrate was treated with lead acetate to remove alginates and proteins. After removal of the precipitate, the excess lead in the filtrate was eliminated by the cautious addition of the required amount of 4N. sulphuric acid solution. It was found that, on an average, 1 c.c. of 4N. sulphuric acid solution for each 200 c.c. filtrate was the minimum volume of acid required. After removal of the last traces of lead sulphate by filtration, the clear filtrate was evaporated to about 2.500 c.c., freed from precipitated salts and further concentrated under reduced pressure to 200 c.c.

Warm methanol (1 litre) was added to this solution precipitating a granular material which was removed and washed with three portions, 150 c.c. each, of the same solvent. Ether (600 c.c.) was added to the methanolic filtrate, more impurities being precipitated and removed by centrifugation. These impurities gave a strongly positive naphthoresorcinol test indicating the presence of hexuronic acid(s).

The solution was concentrated to 100c.c., an equal volume of alcohol was added and the precipitated impurities were removed. This process was

repeated until no further precipitation occurred with alcohol. The final clear filtrate was taken to dryness giving a syrup (31.5g.), $[\alpha]_D^{16°}$ - 66° (c, 1.5 in water).

An attempt was made at this stage to crystallise a portion of the fucose. The thick syrup (5g.), after prolonged drying to reduce the water content, was dissolved in absolute alcohol (20cc.) and the solution kept at 0°. Partial crystallisation occurred after several days, the fucose obtained being impure and in poor yield, $[\alpha]_D^{16°}$ - 69° (c, 2.0 in water). The purity was judged from the specific rotation, assuming the equilibrium $[\alpha]_D$ of pure L-fucose to be -76.0° in water (100).

Preparation of L-Fucose phenylhydrazone.

The thick syrupy crude fucose (26g.) was dissolved in absolute alcohol (40cc.), glacial acetic acid (8 drops) and phenylhydrazine (6cc.) were added; the clear, viscous solution was kept at 0°, crystals appearing within 30min. The precipitation of the phenylhydrazone was complete after 18 hours, a solid yellow mass being obtained. The latter was filtered and carefully washed thrice with 6cc. portions of absolute alcohol cooled at 0°, followed by two washings with 5cc. portions of ether. The crude phenylhydrazone was badly contaminated, but the repeated washings yielded 12g. of pure, colourless fucose phenylhydrazone. Processing of the mother liquors

afforded further quantities of this derivative, which were also purified.

The pure phenylhydrazone (12g.) was decomposed by heating at 100° for 3 hours with an equal weight of benzaldehyde in an aqueous alcoholic solution (75cc.:60cc.) and in the presence of a small quantity of animal charcoal. After cooling, the benzaldehyde phenylhydrazone was removed by filtration through a thin layer of decolourising charcoal. The filtrate was extracted thrice with chloroform (100cc.) in order to remove benzoic acid and excess benzaldehyde. The aqueous layer was concentrated *in vacuo*, crystallisation beginning fairly quickly and being completed by storage at 0° over 24 hours.
Yield: 8.1g. pure fucose, $[\alpha]_D^{16°}$ -70.2° (c, 2.0 in water), m.p. 140-142°.

In the subsequent repeated preparations of fucose by the above method, which is essentially that described by Tollens et al. (101) as modified by Clark (102), the recommendations made by Hudson et al. (100) were adopted. Thus, the seaweed hydrolysate was worked up as before but the addition of lead acetate was omitted. Also, in some experiments, neutralisation of the sulphuric acid in the hydrolysate was effected by the addition of calcium carbonate followed by the necessary amount of calcium hydroxide. - The preliminary cold sulphuric acid solution washing was sometimes omitted as it was found to extract as much as 20% of the fucose present

in the seaweed. On the other hand, preliminary cold acid treatment of the weed removes the bulk of the mineral matter, which is therefore eliminated before hydrolysis and does not subsequently contaminate the fucose phenylhydrazone precipitate. Also, cold acid extracts most of the mannitol present in the seaweed, thus rendering the concentrated hydrolysate less viscous and the hydrazone is more readily filtered from the mother liquor. It seemed, therefore, on the balance desirable to treat the weed with cold acid before hydrolysis.

The addition of animal charcoal to the neutralised hydrolysate considerably lightened the colour of the latter when allowed to stand for 18 hours.

Representative yields for the preparation of crystalline L-fucose by processing the whole weed were: Fucus vesiculosus (500g.) → syrupy fucose (42g.) → crude, badly contaminated fucose phenylhydrazone(89g.) → pure fucose phenylhydrazone(10-15g.) → crystalline L-fucose(6-7g.) m.p.140-142 , $[\alpha]_D^{16°}$ -70.2° (c, 2.0 in H_2O).

2. Preparation of L-fucose from Fucoidin.

Fucoidin, a polyfucose ethereal sulphate occurring as a cell-wall mucilage in all Phaeophyceae, was found to be a more suitable source of L-fucose than the whole weed. As no especially good source of fucoidin exists, Fucus vesiculosus was again employed because of its ready availability.

Preparation of Fucoidin.

The dried, milled weed (200 g.) was heated with water (2000 c.c.) at 100° for 8 hours (pH of mixture 5.8) with stirring. Water was used as the extracting liquid, as with it there is less chance of degrading the fucoidin by partial hydrolysis than with acid. After extraction, the weed residue was centrifuged, washed twice with water (800 c.c. portions) and discarded. Repeated experiments showed that more efficient extraction could be achieved by increasing the water:weed ratio, the extraction time, or the number of extractions. As a rule, 2000 c.c. water were used per 200 g. seaweed, three extractions of 6-9 hours each being adequate.

The combined centrifugate and washings from the extraction were evaporated at 50°/20 mm. to dryness and the brown glass (approx.110 g.) redissolved in water (1000 c.c.) The solution was treated with alcohol to 20% (v/v) concentration, and the brown precipitate (containing most of the soluble alginate) discarded. The alcoholic centrifugate was further treated with alcohol to 60% (v/v) concentration and crude fucoidin was centrifuged and isolated as a brown powder in approx. 50% yield.

Purification of crude fucoidin.

Crude fucoidin (50 g.) was dissolved in water (500 c.c.), 40% formaldehyde (18 c.c.) added, the solution evaporated *in vacuo* at 50°, and the glass

obtained dried for 6 hours at 50°/10mm. The glass was extracted with hot water (1000c.c.) and the insol-:uble residue centrifuged, washed with hot water (300c.c. of washings being collected) and discarded. The formation of this insoluble residue is due to the addition of formaldehyde and effects considerable purification which is, however, accompanied by an approx. 20% loss of fucoidin. The centrifugate and washings were treated with sodium chloride (3g.), as a help to coagulation, and alcohol was added to 70% (v/v) concentration, when fucoidin is precipitated, centrifuged, washed with alcohol and ether, dried and ground to an almost colourless powder (30g.).

Pure fucoidin (2g.) was dissolved in water and treated a second time with 40% formaldehyde (0.75c.c.) as above: no insoluble precipitate was obtained showing the fucoidin prepared as above to be pure. The latter was recovered unchanged on precipitation with alcohol.

Preparation of L-Fucose from Fucoidin by the Phenylhydrazone procedure.

Purified fucoidin (10g.) was hydrolysed with 0.5N. sulphuric acid solution (400c.c.) at 100° for 4 hours; the hydrolysate was neutralised by passage through a resin column (Amberlite IR-4B-OH) and the effluent combined with the aqueous washings (200c.c.) of the resin. Evaporation under reduced pressure at 50° gave a yellow glass which was dissolved in water

(40c.c.); alcohol (400c.c.) was added, the brown sticky precipitate removed by centrifuging and the centrifug-:ate evaporated to a syrupy glass (5g.).

The glass was dissolved in water (6c.c.) and alcohol (45c.c.), phenylhydrazine (5.5g.), and glacial acetic acid (1c.c.) added, the solution being kept at 0° for 48 hours. The crystalline fucose phenylhydr-:azone was removed by filtration, washed twice with alcohol (10c.c.) and dried *in vacuo* over phosphoric oxide to an off-white solid (2.9g.) (*Yield*: 45%)(Based on a fucose ($C_6H_{12}O_5$) content of 41% in fucoidin), m.p.168-170°.

The phenylhydrazone was decomposed by suspending the solid in water (65c.c.), benzaldehyde (1.7g.) added, and the mixture heated at 90° for 60 min. with frequent stirring. After cooling to 0°, the benzal-:dehyde phenylhydrazone was removed by filtration, the filtrate extracted thrice with chloroform (10c.c. portions) to remove benzoid acid and excess benzalde-:hyde and evaporated *in vacuo* to a pale yellow syrup (2.05g.). A trace of absolute alcohol was added and the syrup seeded, when it crystallised quickly. After 4 days at 0° the crystals were filtered, washed with absolute alcohol and dried (1.5g.), m.p.132-136°. The low m.p. shows the fucose to be contaminated with mineral matter; moreover the yield from fucoidin is about 40%.

Preparation of L-Fucose from Fucoidin using Ion Exchange Resins.

Since the most serious loss of yield in the

above described preparation of fucose is at the formation of the phenylhydrazone, where about 50% of the fucose in the hydrolysate is lost, it was attempted to crystallise fucose directly from the fucoidin hydrolysate after removal of salts and the acid with anion and cation exchange resins.

Purified fucoidin (10g.) was hydrolysed with 0.5N. sulphuric acid solution (800c.c.) at 100° for 4 hours, and the solution centrifuged to remove a small brown precipitate. The pale yellow centrifugate was passed through a resin column (Amberlite IR-100-H) to remove cations, and the effluent neutralised by allowing it to percolate through a second (larger) resin column (Amberlite IR-4B-OH). The effluent and aqueous washings (1000c.c.) of the resins were combined and evaporated under reduced pressure at 50° to a syrup. The latter was dissolved in water (40c.c.), alcohol (400c.c.) added, and the brown precipitate removed by centrifuging. Further impurities were removed by concentrating the centrifugate again to dryness, extracting the residual syrup with absolute alcohol (100c.c.) and centrifuging from the undissolved material, this process being repeated until no further precipitation of the syrup with alcohol occurred. Finally, the solvent was evaporated to give a yellow syrup (4.5g.) which was dissolved in the minimum amount of absolute alcohol, seeded and the solution kept at 0°. Crystallisation

set in fairly quickly and, after 4 days, the fucose crystals were filtered, washed twice with alcohol(2c.c.) and dried (2.3g.).

$[\alpha]_D^{16}$ -69° (c, 2.5 in water), m.p. 130-133°.

Although this product was slightly less pure than that obtained by the phenylhydrazone method, the yield has been increased from an average of 40% to an average of 60% (from fucoidin).

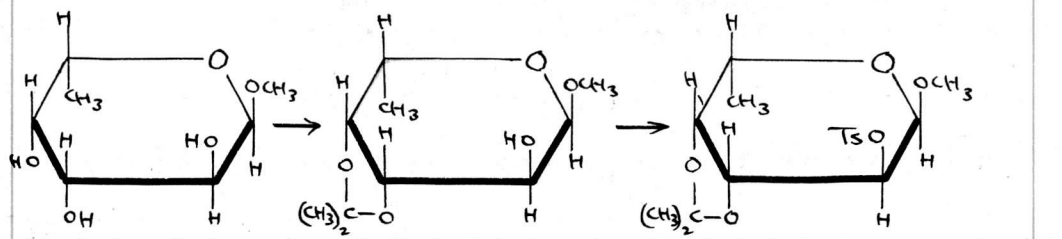
In all subsequent preparations of L-fucose the sugar was obtained directly from hydrolysed pure fucoidin by means of anion and cation exchange resins.

Recrystallisation of Fucose.

Some purification (judged by a slight increase in the optical rotation) was effected, as recommended by Hudson (loc.cit.) by dissolving the fucose in the minimum amount of water, evaporating to dryness, dissolving the yellow syrup in the minimum amount of absolute alcohol and storing at 0°. Crystallisation occurred at times spontaneously, and at others on seeding. The washed and dried crystals had $[\alpha]_D^{16}$ -72° (c, 2.2 in water).

The yellow colour of the syrup (and the occasional yellowish tinge of the crystals) could be removed by heating the aqueous solution of the syrup with a little charcoal. Filtration and evaporation yielded a colourless syrup which, when treated as above with absolute alcohol, gave colourless crystals of L-fucose, m.p. 140-142°.

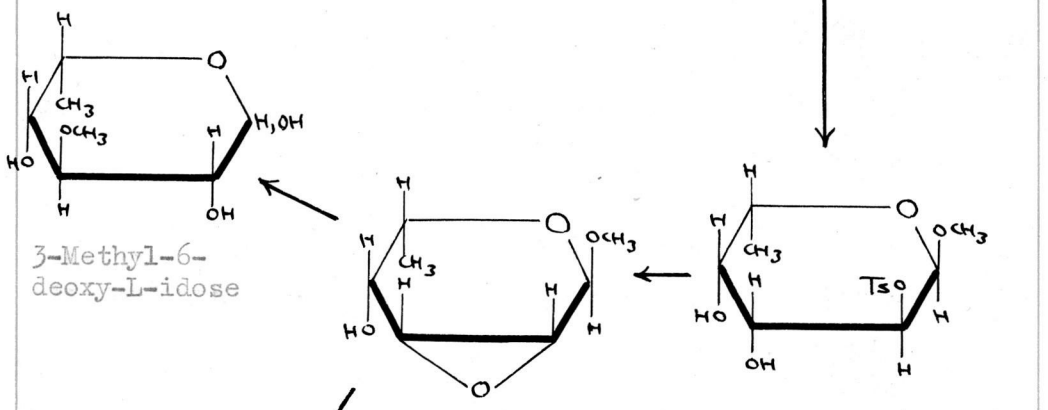
SYNTHESIS OF 3-METHYL-6-DEOXY-L-IDOSE, 6-DEOXY-L-IDOSE AND OF 2:4-DIMETHYL-L-FUCOSE.



Methyl α-L-fucoside

Methyl 3:4-isopropylidene α-L-fucoside

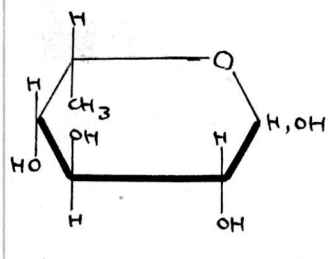
Methyl 3:4-isopropylidene 2-toluene-p-sulphonyl α-L-fucoside



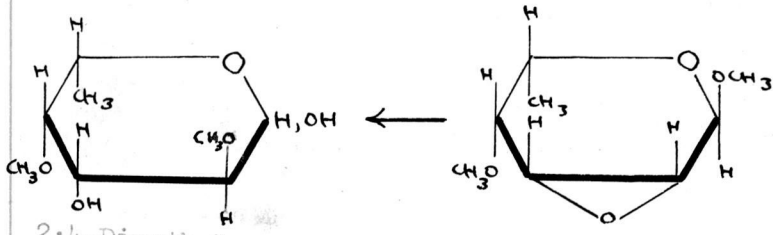
3-Methyl-6-deoxy-L-idose

Methyl 2:3-anhydro-α-L-taloside

Methyl 2-toluene-p-sulphonyl-α-L-fucoside



6-Deoxy-L-idose



2:4-Dimethyl L-fucose

Methyl 2:3-anhydro-4-methyl α-L-taloside

Methyl ∝-L-Fucoside

L-Fucose(2.40g.), m.p.145°, $[\alpha]_D^{14°}$ -72°(c, 2.02 in water), was dissolved in dry methanolic hydrogen chloride solution (90c.c.; 2.5%) and heated at 70° under reflux until non-reducing to Fehling's solution (4 hours). After cooling, the solution was neutralised with silver carbonate; the latter was then removed by filtration and extracted with hot chloroform four times. The combined filtrate and extracts were taken to dryness under diminished pressure at 30° affording crystalline methyl ∝-L-fucoside(2.01g.) m.p. 160-161°, $[\alpha]_D^{16°}$ -198° (c, 1.0 in water).

In a second experiment, L-fucose(2.50g.) was dissolved in dry methanol (30c.c.) and heated under reflux at 70° in the presence of cation exchange resin (Amberlite IR-100-H; 3.0g.) until the solution became non-reducing to Fehling's solution (10 hours). The resin was removed by filtration and washed with warm methanol (6 x 5c.c.). Evaporation of the combined washings and filtrate gave the glycoside(2.0g.), m.p.159-160°, $[\alpha]_D^{14°}$ -200 (c, 1.5 in water).

Methyl 3:4-isoPropylidene-∝-L-fucoside.

Methyl ∝-L-fucoside(2.0g.) was dissolved in dry acetone(130c.c.), acetaldehyde (4 drops) and anhydrous copper sulphate(28g.) were added and the mixture was vigorously shaken for 120 hours. Subsequent treatment as described in the isolation of this

derivative of rhamnose (p. 31) gave a syrup which distilled at 95° (bath temperature)/0.01mm. Yield: 1.85g.; $n_D^{16°}$ 1.4621; $[\alpha]_D^{14°}$ -160°(c, 1.1 in water).

In a second experiment, methyl α-L-fucoside (2.35g.) gave the isopropylidene compound in better yield(2.50g.), distilling at 110-112°(bath temperature) /0.07mm., $n_D^{16°}$ 1.4630.

Methyl 3:4-isoPropylidene-2-toluene-p-sulphonyl-α-L-fucoside.

Methyl 3:4-isopropylidene-α-L-fucoside(3.22g.) was dissolved in dry pyridine(30c.c.) and finely powdered toluene-p-sulphonyl chloride (6g.) was added in half-gram portions, the solution being cooled in ice-water and frequently stirred; it was then kept at 15° for 40 hours and at 25° for a further 8 hours. The originally orange colour of the solution increasingly deepened until it became a dark red, when big crystals of the toluene-p-sulphonyl compound appeared; large feathery crystals of pyridine hydrochloride were also deposited. The mixture was poured onto crushed ice with stirring, when a crystalline solid was obtained along with an amorphous powder. The crystals and powder were removed by filtration. Extraction of the filtrate with chloroform (4 x 100c.c.) as described on p.32 afforded a further 75mg. of amorphous powder. Total yield: 3.61g. Large crystals, m.p.200°; small crystals and amorphous powder,

m.p.185-186°(recryst. dry methanol); $[\alpha]_D^{16°}$ -146°(c, 1.0 in chloroform).

Found: C, 54.8; H,6.5; OMe, 8.45. Calc. for $C_{17}H_{24}O_7S$: C, 54.8; H, 6.45; OMe, 8.3%.

Methyl 2-toluene-p-sulphonyl ∝-L-fucoside.

Crystalline methyl 3:4-isopropylidene-2-toluene-p-sulphonyl-∝-L-fucoside(3.60g.) dissolved in dry methanolic hydrogen chloride solution(80cc.;1%) was heated at 70° under reflux for 75 minutes. The solution was then neutralised with silver carbonate and, after filtration, the silver residues were extracted with alcohol (4 x 25cc.). The combined extracts and filtrate were taken to dryness yielding crystalline methyl 2-toluene-p-sulphonyl-∝-L-fucoside(3.31g.), m.p. 159-160°, $[\alpha]_D^{16°}$ -85 (c, 1.0 in chloroform).

Found: C,51.45; H, 6.39. Calc. for $C_{14}H_{20}O_7S$. C,50.6; H, 6.02.

Methyl 2:3-anhydro-6-deoxy-∝-L-taloside.

Crystalline methyl 2-toluene-p-sulphonyl-∝-L-fucoside(3.30g.) was dissolved in ethanol(30cc.) and titrated with sodium hydroxide solution at 75° until permanently pink to phenolphthalein(9.00cc.;2M.). The solution was then allowed to stand at 15° for 18 hours in the presence of a slight excess of alkali. Large crystals of sodium toluene-p-sulphonate which had deposited overnight were removed by filtration

and the filtrate taken to dryness yielding a crystal-:line solid admixed with syrup. This product was extracted with cold, dry ethyl acetate (6 x 25c.c.) and with boiling ethyl acetate(20c.c.); removal of the solvent from the combined extracts afforded crystalline methyl 2:3-anhydro-6-deoxy-α-L-taloside(1.70g.), m.p.95-97°(recryst. from ethanol as very light hair-like wavy strands), $[\alpha]_D^{16°}$ -88°(c, 2.0 in water).

Found: C,52.45; H, 7.31. $C_7H_{12}O_4$ requires C,52.5; H, 7.5%.

This substance is extremely volatile and very soluble in methanol, ethanol, acetone, chloroform and petroleum ether.

Alkaline hydrolysis of methyl 2:3-anhydro-6-deoxy-α-L-taloside.

Crystalline methyl 2:3-anhydro-6-deoxy-α-L-taloside(0.35g.) was dissolved in dry methanol(35c.c.) containing metallic sodium(0.35g.) and the solution was heated at 75-80° under reflux for 19 hours. Subsequent treatment as described for the fission of methyl 3:4-anhydro-6-deoxy-α-L-taloside(p.37) gave a yellow syrup(0.32g.), $[\alpha]_D^{20°}$ -104°(c, 0.8 in water). This syrup was hydrolysed at 100° with aqueous sulphuric acid (15c.c.; 4%) until the optical rotation became constant ($[\alpha]_D^{18°}$ -13°; 3 hours). This syrup was neutralised by being allowed to percolate through a resin column (Amberlite IR-4B-OH). Removal of the solvent from the effluent gave a colourless syrup (0.21g.) which, on chromatographic examination (with solvent (I)

as mobile phase) showed two spots. The slower one, R_G 0.50, was presumed to be 2-methyl-L-fucose. The faster spot, R_G 0.75, M_G 0.80 was identical with that produced by 6-deoxy-3-methyl-L-idose obtained by a similar series of operations from the sodium methoxide scission of the anhydro ring of methyl 3:4-anhydro-6-deoxy-α-L-taloside.

Subsequent preparations also gave traces of free fucose.

Separation by chromatographic adsorption.

The above mixture of sugars(0.20g.) was separated into its components on a column of powdered cellulose (108). The solvent employed for elution was purified light petroleum ether (b.p.110-120°)-n-butanol saturated with water (50:50, v/v).

Fraction I (0.150g; 75%), a pale yellow syrup, 6-deoxy-3-methyl-L-idose, had $[\alpha]_D^{16°}$ -14°(c, 0.7 in water), (Found: OMe, 17.5; $C_7H_{14}O_5$ requires OMe,17.4%), and produced a single spot, R_G 0.75, on the paper chromatogram using solvent (I) as the mobile phase. This syrup was mixed with 6-deoxy-3-methyl-L-idose ($[\alpha]_D^{16°}$ -14°; c, 0.06 in water) obtained from methyl 3:4-anhydro-6-deoxy-α-L-taloside (p.39) and the mixture run on a paper chromatogram with the same eluant as above: a single spot, R_G 0.75, was obtained. These two derivatives, having the same optical rotation and producing on the paper chromatogram the same single spot, R_G 0.75, also revealed identical spots M_G 0.80 on filter paper ionophoresis.

Fraction I (50mg.) on heating in ethanol(10cc.) with aniline(0.2cc.) at 80° for 5 hours gave a crystal-:line anilide, m.p.62-3°(recryst. light petroleum, (b.p.40-60°) undepressed on admixture with 6-deoxy-3-methyl-N-phenyl-L-idosylamine, m.p.62-3°(p.39), obtained from the 3:4-anhydroderivative.

Fraction I(60mg.) was converted into the phenyl-osazone according to the method described on p.40 . A crystalline osazone was obtained and had, after recrystallisation from aqueous ethanol, m.p.122-3°, undepressed on admixture with 6-deoxy-3-methyl-N-phenyl-L-idosazone.

Fraction II(0.030g.;15%), $[\alpha]_D^{16°}$ -22°(c, 1.0 in water, R_G 0.50 with solvent (I) as eluant, was presumed at first to be 2-methyl-L-fucose. However, nucleation with synthetic 2-methyl-L-fucose failed to induce crystallisation, and a mixture of the two substances on a paper chromatogram with the same eluant as above revealed two spots: synthetic 2-methyl-L-fucose, R_G 0.59, and a slightly slower spot, R_G 0.50. Paper ionophoresis showed much greater difference in the mobility of the two substances. 2-Methyl-L-fucose had M_G 0.33, while the syrup from fraction II had M_G 1.0, and L-fucose had M_G 0.92.

Admixture of this fraction with 6-deoxy-L-idose isolated from the fission products of methyl 3:4-anhydro-L-taloside and chromatographic analysis of the mixture showed a single spot, R_G 0.50, with solvent (I)

as eluant.

Recovery from the chromatographic separation: 90%.

Synthesis of 2-methyl-L-fucose for comparison.

Methyl 3:4-isopropylidene-α-L-fucoside(0.27g.) was methylated four times with methyl iodide and silver oxide to give a yellow syrup(0.30g.). The isopropylidene residue was then removed by boiling with dry methanolic hydrogen chloride solution(1%) as above and the product(0.22g.) was hydrolysed with aqueous sulphuric acid(15c.c.;4%) as described before (p.86), neutralisation being effected by percolation of the hydrolysate through a resin column (Amberlite 1R-4B-OH). Evaporation of the solvent gave crystalline 2-methyl-L-fucose(0.18g.), m.p.150-152°, $[\alpha]_D^{17°}$ -73°(c, 2.7 in water), producing a single spot, R_G 0.59, on the paper chromatogram with solvent (I) as eluant.

Methyl 3:4-anhydro-4-methyl-α-L-taloside.

Due to its sensitivity towards changes in pH, methyl 2:3-anhydro-α-L-taloside was methylated with silver oxide and methyl iodide. The extent of methylation was followed by micro Zeisel determination of methoxyl content after each methylation. After four treatments with Purdie's reagents a completely crystalline product (0.95g. from 1.10g.) was obtained.

Recrystallisation from acetone-light petroleum (b.p.40-60°) gave long needles, m.p.108-110°, $[\alpha]_D^{16°}$ ± 0.0°(c, 0.7 in ethanol, acetone and chloroform).

Found: C,54.7; H,8.1; OMe,35.0. Calc.for $C_8H_{14}O_4$ C,55.2; H,8.1; OMe,35.6%.

Hydrolysis of methyl 2:3-anhydro-4-methyl-α-L-6-deoxy-taloside with sodium methoxide.

Crystalline methyl 2:3-anhydro-4-methyl-α-L-taloside(0.90g.) dissolved in dry methanol(80cc.) containing metallic sodium(0.8g.) was heated under reflux at 80° for 19 hours. The product was isolated as described previously for the unmethylated derivative(p. 37). A mobile syrup(0.70g.) was obtained; this was hydrolysed with aqueous sulphuric acid (30cc.;4%), neutralisation being effected by allowing the hydrolysate to percolate through a resin column (Amberlite 1R-4B-OH). Removal of the solvent afforded a reducing syrup (F) (0.41g.), $[\alpha]_D^{18°}$ -15°(c, 2.4 in methanol), which showed a single spot, R_G 0.80, on the paper chromatogram with solvent (I) as eluant; (Found: OMe,31.8; $C_8H_{16}O_5$ requires OMe, 32.3%).

Characterisation of syrup (F).

(a) Demethylation. Syrup (F) (50mg.) was treated with hydriodic acid (sp.g.1.7;2cc.) as described on p.45 . After deionisation a syrup was obtained which produced on the paper chromatogram a single spot, R_G 0.21, with solvent (I) as the eluant, ~~indistinguish-~~

indistinguishable from that given by L-fucose.

(b) Methylation. Syrup (F) (0.1g.) was converted into the glycoside and methylated with Purdie's reagents; hydrolysis with 4% aqueous sulphuric acid afforded a syrup which produced on the paper chromatogram a single spot, R_G 0.95, with solvent (I) as eluant, corresponding to that obtained from authentic 2:3:4-trimethyl-L-fucose.

(c) Syrup (F) (0.235g.) was converted into the glycoside by refluxing with methanolic hydrogen chloride solution (24cc.; 2%) until non-reducing towards Fehling's solution (3 hours). This afforded a syrupy glycoside, $[\alpha]_D^{16°}$ -30°(c, 1.5 in ethanol). This syrup was methylated with Purdie's reagents (5 treatments) when the product crystallised spontaneously on removal of the solvents (0.15g. pure crystals) m.p. 92-95°, mixed m.p. with authentic (especially prepared) methyl 2:3:4-trimethyl-α-L-fucoside (m.p. 94-97°) 91-96°, $[\alpha]_D^{16°}$ -200°(c, 1.0 in water). [cf. Smith(116) who records m.p. 85-92° and $[\alpha]_D^{16°}$ -196°(c, 0.5 in water) for methyl 2:3:4-trimethyl-α-L-fucoside].

Found: C,54.05; H,9.06; OMe,55.4. $C_{10}H_{20}O_5$ requires C,54.55; H,9.09; OMe,56.3%.

These results indicate a fucose configuration for (F) which would then be 2:4-dimethyl-L-fucose.

Synthesis of methyl 2:3:4-trimethyl-α-L-fucoside and 2:3:4-trimethyl-L-fucose by direct methylation of methyl α-L-fucoside.

L-Fucose(2.0g.) was heated at 70° in dry methan-

olic hydrogen chloride solution (90cc.; 2%) until non-reducing towards Fehling's solution (4 hours). The methyl α-L-fucoside(2.0g.), m.p.157-158°, was methylated by four treatments with methyl iodide and silver oxide, a small volume of dry methanol being added at the first treatment to effect complete dissolution of the crystals. Removal of the solvents after the fourth methylation afforded a product which crystallised spontaneously, methyl 2:3:4-trimethyl-α-L-fucoside(2.01g.), m.p.94-97°, $[\alpha]_D^{16°}$ -198°(c,1.5 in water).

Found: C, 54.18; H,9.20; OMe,55.1. $C_{10}H_{20}O_5$ requires C, 54.55; H,9.09; OMe,56.3%.

Methyl 2:3:4-trimethyl α-L-fucoside(50mg.) was hydrolysed with aqueous sulphuric acid (N.,10cc.) at 100° for 3 hours giving 2:3:4-trimethyl-L-fucose(48mg.) as a colourless, reducing syrup, $[\alpha]_D^{16°}$ -110°(c, 0.1 in water). On the paper chromatogram, with solvent (I) as eluant, this product gave a single spot, R_G 0.95.

DISCUSSION.

Crystalline L-fucose was prepared from Fucus vesiculosus in the first instance by processing the whole weed according to the method of Tollens et al. (101) as modified by Clark(102) and by Hudson et al. (100). The preliminary wash with cold aqueous sulphuric acid was on the whole deemed necessary since, in spite of its removing nearly 20% of the fucose in the weed, it achieves the useful elimination of the major portion of inorganic salts, of mannitol and other soluble matter. The removal of these materials enables a much purer fucose phenylhydrazone to be obtained and hence ash-free fucose.

More satisfactory yields were obtained by using the polysaccharide fucoidin as a source of fucose. The optimum conditions for the extraction of fucoidin from Fucus vesiculosus were found to be stirring for 6-9 hours at 80-100° one part (by weight) of the dried milled weed with ten parts (by volume) of water. Water as the extracting liquid made it sometimes difficult to separate the weed residue from the solu-:tion, but it was preferred to acid as with the latter there was greater danger of degrading the fucoidin. An increase in the water-weed ratio, the extraction time and the number of extractions led to slightly more efficient recovery of fucoidin, but, as a rule, three extractions as above were found to remove about 80% of the fucoidin present.

Evaporation to dryness of the aqueous extracts, dissolution in water, and fractional precipitation with ethanol at 20% and 60% (v/v) concentration afforded crude fucoidin in 50-60% yield. (The 60% fraction is crude fucoidin). Pure fucoidin was obtained by treatment with formaldehyde and separation from the resulting insoluble compound. This treatment entails a 20% loss of fucoidin, but the degree of purification thereby achieved renders this worthwhile.

Purified fucoidin was employed as a source of fucose in the greater number of preparations, two methods being used. Hydrolysis of the polysaccharide with aqueous sulphuric acid, followed by neutralisation by percolation through a resin column (Amberlite 1R-4B-OH), and treatment with phenylhydrazine afforded fucose phenylhydrazone in 45% yield, from which, after decomposition, fucose was obtained in 50% yield (from fucoidin).(Based on a 41% fucose ($C_6H_{12}O_5$) content of of fucoidin).

Ion exchange resins were found to be the best means of obtaining crystalline fucose directly from hydrolysed fucoidin, thus avoiding the intermediate phenylhydrazone step, where the most serious loss of yield occurs. Hydrolysis of the polysaccharide with aqueous sulphuric acid followed by deionisation with successive percolations through resin columns (Amberlite 1R-100-H and 1R-4B-OH) afforded an effluent which was purified by ethanol precipitation. L-fucose,

$[\alpha]_D^{16°}$ -70°(c, 2.5 in water) crystallised from an ethanolic solution in approx. 60% yield. (Based on a 41% fucose content in fucoidin).

Using the m.p. and specific rotation values as criteria it is seen that fucose crystallised directly from the fucoidin deionised hydrolysate is slightly less pure than the sugar obtained via the phenylhydrazone; this however is compensated by the increase in yield from an average of 40% to an average of 60%. Derivatives prepared from L-fucose obtained by any of the above described methods gave equally good analytical figures and possessed comparable constants.

Methyl ∝-L-fucoside was obtained crystalline by both the methanolic hydrogen chloride and the cation exchange method. The latter method was extensively employed for the preparation of glycosides in the fucose series because of the small quantities available.

Condensation of the fucoside with acetone was achieved under the same conditions that were found to be effective for the preparation of the corresponding rhamnose derivative. In the present instance there are two cis - situated hydroxyl groups at C_3 and C_4 and condensation with acetone took place at these positions, methyl 3:4-isopropylidene ∝-L-fucoside being obtained.

Since the only remaining free hydroxyl group is now located at C_2, toluene-p-sulphonation of the iso

propylidene compound can only have resulted in the production of methyl 3:4-*iso*propylidene-2-toluene-*p*-sulphonyl-α-L-fucoside. Large crystals of this derivative were deposited from the pyridine solution along with the customary feathery, water-soluble pyridine hydrochloride crystals, and could be separated before any further treatment of the alkaline solution.

Removal of the *iso*propylidene residue was effected by means of refluxing 1% methanolic hydrogen chloride solution, the pure methyl 2-toluene-*p*-sulphonyl α-L-fucoside being also obtained crystalline. The fact that all the intermediates in this series of reactions were crystalline led to a very high degree of purity being maintained.

The presence of a free hydroxyl group on C_3, trans-situated to the toluene-*p*-sulphonyl group at C_2, made easy removal of this group possible and treatment with sodium methoxide, the calculated volume of aqueous 2M. reagent being added exactly in the presence of phenolphthalein, resulted in the removal of the toluene-*p*-sulphonyl group with Walden inversion on C_2 and the formation of crystalline methyl 2:3-anhydro-6-deoxy-α-L-taloside. This material proved exceedingly volatile and great care had to be exercised to prevent its complete loss on removal of solvents. It was necessary to carry out all extractions and evaporations at room temperature. A further characteristic of this compound is its crystalline aspect which

is in the form of very light wavy filiform strands.

Fission of the anhydro-ring was achieved by means of the alkaline reagent sodium methoxide and this was followed by hydrolysis with aqueous sulphuric acid. Depending on the side of the oxide ring breaking, 6-deoxy-3-methyl-L-idose and 6-deoxy-2-methyl-L-galactose (2-methyl-L-fucose) would be expected to form. In actual fact a single main product was obtained in 75-80% yield, and this was shown to be 6-deoxy-3-methyl-L-idose by direct comparison of its constants and those of the derived crystalline anilide and phenylosazone with those of the same compound obtained under similar conditions from methyl 3:4-anhydro-6-deoxy-α-L-taloside(p. 39) and of its anilide and osazone. Repeated fissions of this 2:3-anhydroring failed to yield any other main product; traces of free L-fucose and up to 15% of a substance of intermediate R_G 0.50 were invariably obtained. The latter compound was at first taken to be the other possible fission product, 2-methyl-L-fucose; it failed to crystallise however on nucleation with authentic 2-methyl-L-fucose (especially prepared) and a mixture of the two substances analysed chromatographically showed two distinct spots, R_G 0.50 and R_G 0.59. Ionophoresis gave M_G 1.0 and M_G 0.33 for the two substances, respectively. The specific rotation $[\alpha]_D^{16°}$ -22° was quite different from that of 2-methyl-L-fucose, $[\alpha]_D^{18°}$ -73°, but more closely resembles that of 6-deoxy-L-idose. Admixture with

suspected 6-deoxy-L-idose obtained in the L-rhamnose series of reactions(p. 36) gave a single spot on chromatographic analysis with solvents (I) and (II) as eluants.

It is difficult to understand why no trace of 6-deoxy-2-methyl-L-galactose could be found in the scission products from the methyl 2:3-anhydro-L-taloside as both possible derivatives were isolated from the fissions of methyl 2:3-anhydro-D-lyxoside and of methyl 3:4-anhydro-L-taloside. Gyr and Reichstein(115) recorded the isolation of a single product, 4:6-benzylidene 3-methyl-α-D-idoside, in excellent yield from the action of sodium methoxide on methyl 2:3-anhydro-4:6-benzylidene-α-D-taloside, and Sorkin and Reichstein(117) obtained methyl 4:6-benzylidene 2-methyl-α-D-idoside as the sole product from methyl 2:3-anhydro-4:6-benzylidene α-D-guloside. It may well be, however, that the deciding factor in these last two scissions is the large benzylidene group attached to carbon atoms 4 and 6.

Methylation with Purdie's reagents of the 2:3-anhydrotaloside afforded, after four treatments, crystalline methyl 2:3-anhydro-4-methyl-α-L-taloside. Alkaline scission of the ethylene oxide ring in this compound was again achieved by means of sodium methoxide; hydrolysis of the product with aqueous sulphuric acid showed that the oxide ring had again suffered scission in one direction only, as a single spot, R_G 0.80, was obtained on the paper chromatogram with (I)

as the mobile phase. This product on demethylation showed after heavy spotting a single spot corresponding to L-fucose, R_G 0.21 with solvent (I) as eluant. The fully methylated glycoside crystallised and had constants which agreed with those found for methyl 2:3:4-trimethyl- ∝-L-fucoside, prepared for the purposes of comparison by direct methylation of methyl ∝-L-fucoside, and also with the constants recorded by Smith(116). Hydrolysis of a portion of the crystalline trimethyl glycoside yielded a syrup which on admixture with authentic 2:3:4-trimethyl-L-fucose produced on the paper chromatogram a single spot, R_G 0.95, with solvent (I) as the mobile phase. The product from the alkaline hydrolysis of methyl 2:3-anhydro-4-methyl- ∝-L-fucoside is thus shown to possess the L-fucose configuration and would therefore be 2:4-dimethyl-L-fucose (6-deoxy-2:4-dimethyl-L-galactose).

The introduction of a methoxyl group in position C_4 appears to have caused a complete reversal of the fission of the oxygen bridges in the epoxide ring. In the unmethylated derivative, methyl 2:3-anhydro-6-deoxy-L-taloside, it is the oxygen bridge nearer C_4 that breaks, but when the hydroxyl group on C_4 is replaced by the -OMe radical then the oxygen bridge further from C_4 breaks. This is analogous with the results obtained from the methylated methyl 3:4-anhydro-6-deoxy-L-taloside and the analogy extends still further in that the entering -OMe radical is trans-

situated to the $-CH_3$ group attached to carbon atom 5.

Although the isolation of a 6-deoxy-3-methyl-L-idose derivative from the unmethylated methyl 2:3-anhydro-6-deoxy-L-taloside is in conformity with the views expressed by Bose et al.(103) that the predominant product from the scission of the epoxide ring in 2:3-anhydrosugars has the entering groups in the polar conformation, the isolation of a 6-deoxy-6-deoxy dimethyl galactose from methyl 2:3-anhydro-4-methyl-L-taloside is in direct disagreement, the entering group being equatorial in conformation.

SUMMARY.

(a) Derivatives from L-Rhamnose.

1. Crystalline methyl 3:4-anhydro-6-deoxy- ∝ -L-taloside has been synthesised from L-rhamnose. Conversion into the glycopyranoside followed by condensation with acetone and treatment with toluene-p-sulphonyl chloride led to the isolation of crystalline methyl 2:3-isopropylidene-4-toluene-p-sulphonyl ∝-L-rhamnoside. Removal of the isopropylidene residue by mild hydrolysis followed by treatment with aqueous sodium hydroxide gave rise to the anhydrodeoxytaloside.

2. Fission of the ethylene oxide ring in the above 3:4-anhydro-6-deoxytaloside with sodium methoxide followed by acid hydrolysis afforded both possible scission products, namely 6-deoxy-4-methyl-L-mannose (4-methyl-L-rhamnose) and 6-deoxy-3-methyl-L-idose. The deoxymannose derivative was crystalline and was identified by comparison of its constants with those recorded in the literature(109) for 4-methyl-L-rhamnose. The second product was shown to be the deoxyidose derivative since an identical compound resulted from the alkaline scission of methyl 2:3-anhydro-6-deoxy-∝-L-taloside; this derivative was further characterised by the isolation of a crystalline anilide and a crystalline phenylosazone.

3./

3. L-Rhamnose was the only product which could be identified with certainty from the fission of the anhydro ring with mineral acid or with barium hydroxide.

4. Methylation of the anhydrodeoxytaloside afforded methyl 3:4-anhydro-6-deoxy-2-methyl-∝-L-taloside as an uncrystallisable syrup, the constants of which are recorded.

5. Fission of the ethylene oxide ring in the methylated anhydrodeoxytaloside by means of sodium methoxide occurred in one direction only, a single scission product being isolated after acid hydrolysis. This product was shown to be 2:4-dimethyl-L-rhamnose by demethylation to L-rhamnose; by the isolation of a crystalline anilide, an X-ray powder photograph of which is identical with an X-ray powder photograph of 2:4-dimethyl-N-phenyl-L-rhamnosylamine; and by conversion into the fully methylated glycoside, nitric acid oxidation of which afforded a trimethoxyglutaric acid, shown to have the L-arabo- configuration by the isolation of crystalline L-arabotrimethoxyglutaric diamide from the dimethyl ester of the acid.

(b) Derivatives from L-Fucose.

1. Crystalline methyl 2:3-anhydro-6-deoxy-∝-L-taloside has been synthesised from crystalline methyl 3:4-isopropylidene-2-toluene-p-sulphonyl ∝-L-fucoside by a route parallel to that followed in the L-rhamnose

series.

2. Fission of the ethylene oxide ring in the above 2:3-anhydro-6-deoxytaloside followed by acid hydrolysis afforded only 6-deoxy-3-methyl-L-idose, since this product proved in every respect identical with the deoxyidose derivative obtained from the alkaline hydrolysis of methyl 3:4-anhydro-6-deoxy-α-L-taloside. It was further characterised by the isolation of the same crystalline anilide and phenylosazone.

3. Methylation of the 2:3-anhydrodeoxytaloside afforded crystalline methyl 2:3-anhydro-6-deoxy-4-methyl-α-L-taloside, the constants and properties of which are recorded.

4. Fission of the ethylene oxide ring in this methylated 2:3-anhydro-6-deoxytaloside, followed by acid hydrolysis, afforded a single scission product: 2:4-dimethyl-L-fucose, which was characterised by conversion into the fully methylated crystalline glycoside. This analysed correctly for a methyl trimethyl-deoxyhexoside and had the same constants as synthetic methyl 2:3:4-trimethyl-α-L-fucoside (prepared directly from L-fucose) and agreed with the constants recorded in the literature(116) for methyl 2:3:4-trimethyl-α-L-fucoside.

P A R T II.

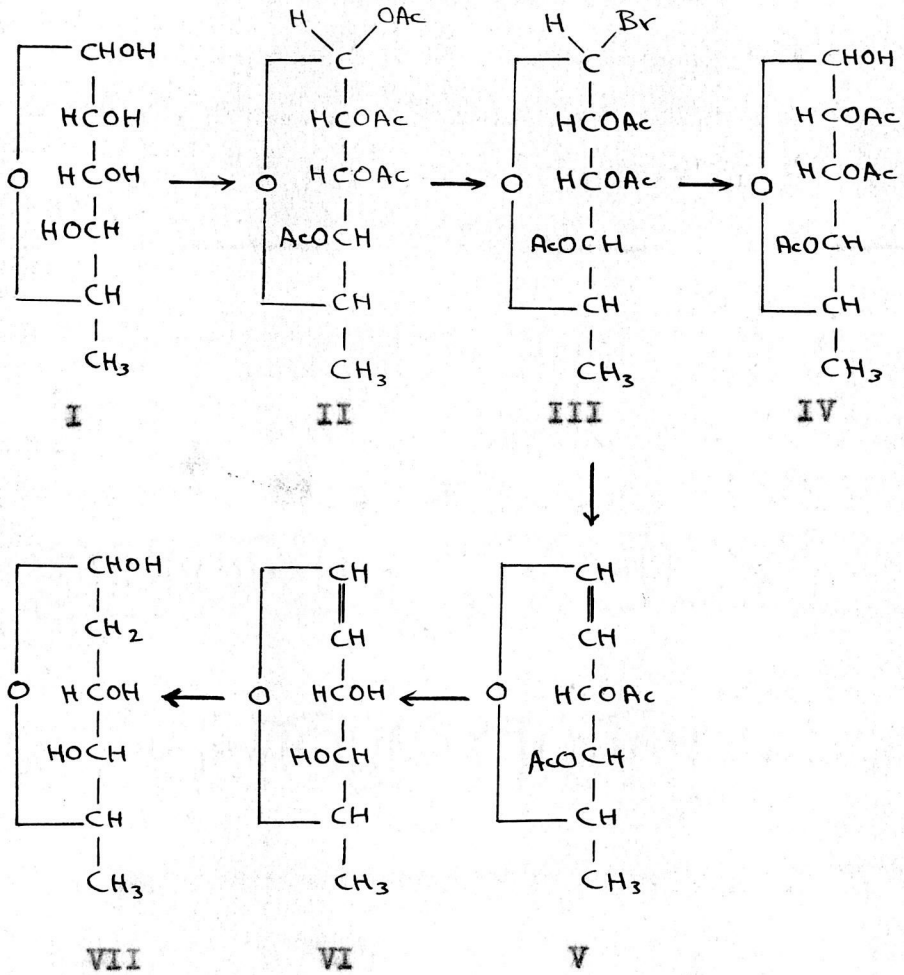
The preparation of dideoxy-derivatives by the treatment of ethylene oxide ring compounds with lithium aluminium hydride.

INTRODUCTION.

Of all deoxy-sugars the naturally occurring 2-deoxy-sugars, found in thymonucleic acids and in the cardiac glycosides have received the greatest attent-:ion. Until 1944 only Fischer's glycal method(61) was known for the synthesis of such substances. This involves the reduction of an acetyl glycosyl halide ("acetobromo") derivative to a glycal; D-glucose was the first sugar to be converted, through the inter-:mediate D-glucal(61,62,63) into 2-deoxy-D-glucose (63,64,65).

This valuable method has, in most cases, led to the synthesis of the desired product and excellent yields were obtained in the preparation of triacetyl glucal (61,66,67); of diacetyl rhamnal(63,68,70); and of diacetyl arabinal(69). The deacetylation of these acetylglycals may be effected by means of aqueous barium hydroxide, but more easily crystallisable products result from deacetylation with ammonia in methanol, alcoholic alkali (e.g. methanolic barium hydroxide), or sodium alkoxides. Cleavage of the double bond of the glycal takes place readily with dilute sulphuric acid and the addition of water is thought to be preceded by the formation of a sulphate ester(64), which in turn gives rise with most sugars

to a crystalline deoxy-derivative. In this way L-rhamnose is converted into crystalline 2-deoxy-L-rhamnose.



Iselin and Reichstein(70) found that the conversion of L-rhamnosetetraacetate(II) into acetyl rhamnosyl bromide(III) is best achieved by hydrogen bromide-acetic acid in the presence of acetic anhydride; in this way the unwanted formation of L-rhamnose triacetate(IV) is minimised. Furthermore, it is preferable not to isolate the acetylrhamnosyl

bromide(III) but to reduce the hydrogen bromide - containing reaction mixture directly at -10° with copper and zinc dust. Under these conditions, diacetyl L-rhamnal(V) is obtained in about 80% yield.

Poor yields were however obtained in the synthes-:is by Fischer's method of triacetyl galactal(71); of diacetyl xylal(72); of diacetyl L-fucal(73)(30% yield); and a bad yield(18%) of diacetyl digitoxoseen) (diacetyl 1:2:6-trideoxy-D-allose)(74). In some instances, as in the attempted conversion of 6-deoxy-3-methyl-D-altrose into the glycal cymaroseen (3-methyl-1:2:6-trideoxy-D-altrose)(75), this method fails to yield the desired product.

The importance of certain deoxy-sugars, partic-:ularly 2-deoxy-D-ribose, has led to many attempts to synthesise this sugar easily and in good yield. Effort has been directed to improve the Fischer glycal method of synthesis and also to develop completely new methods.

An improved glycal method was evolved by Deriaz, Overend, Stacey, Teece and Wiggins(76) for the synthesis of 2-deoxy-D- and 2-deoxy-L-ribose. The usual method, which gave a yield of 2-deoxy-D-ribose of about 5%, was to treat D-arabinal,(obtained by the standard reactions from D-arabinose) with ice-cold normal sulphuric acid solution. These workers found that if the pure, crystalline β-acetyl

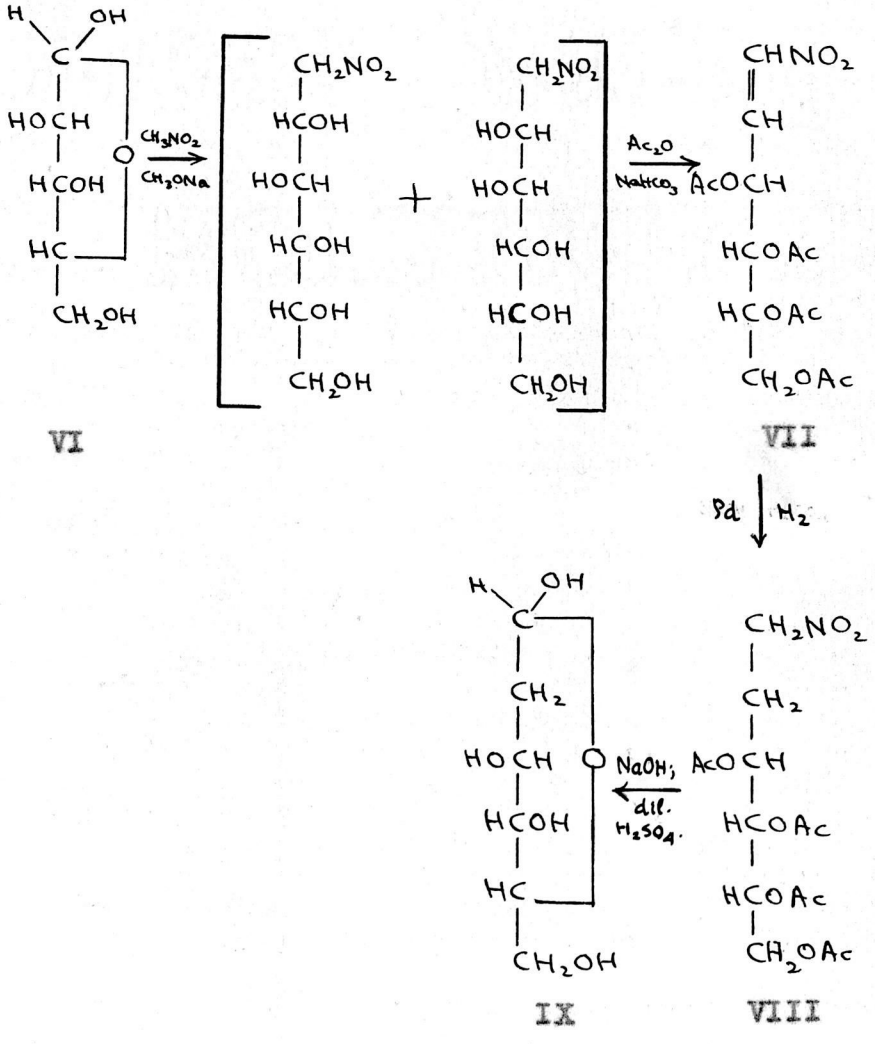
arabinosyl bromide is isolated first and then reduced to the 3:4-diacetyl arabinal with zinc dust in 50% acetic acid solution at -10° in the presence of a few drops of chloroplatinic acid as a catalyst, the overall yield of 2-deoxy-β-L-ribose and 2-deoxy-β-D-ribose could be increased to 10%.

2-Deoxy-D-galactose was similarly obtained by Overend et al.(77) in satisfactory yield. These authors also found that catalytic reduction of 3:4:6-triacetyl-D-galactal followed by deacetylation gave 1:2-dideoxy-D-galactose which had also been obtained by catalytic reduction of D-galactal.

Levene and Tipson(71) and Pigman and Isbell(78) prepared 2-deoxy-D-galactose in poor yield by the original glycal method. The latter workers assumed that on treatment of D-galactal with dilute sulphuric acid solution the deoxy-sugar was formed via an intermediate ester which was hydrolysed when heated with barium carbonate at 60° for a relatively long period. Overend et al.(79) found that 2-deoxy-D-galactose may be obtained in much improved yield by introducing rapid neutralisation of the reaction mixture with barium hydroxide at room temperature. Whether a transient ester is formed is not known, but the latter authors are rather of the opinion that the process described by Pigman and Isbell (loc.cit.) as hydrolysis by barium carbonate was merely slow heterogeneous-

phase neutralisation of sulphuric acid by barium carbonate.

A number of methods designed to supercede the glycal method have also been developed. Fischer and Sowden(80) showed that when D-arabinose(VI) is treat-:ed with nitromethane and an acetylating agent (Sodium acetate) it yields 1-nitro-D-arabo-3:4:5:6-tetra-:acetoxyhex-1-ene(VII) which on reduction gives 1:2-dideoxy-1-nitro-D-arabo-hexityl tetraacetate(VIII). Treatment of this compound with sodium hydroxide followed by dilute sulphuric acid affords 2-deoxy-D-glucose(IX).

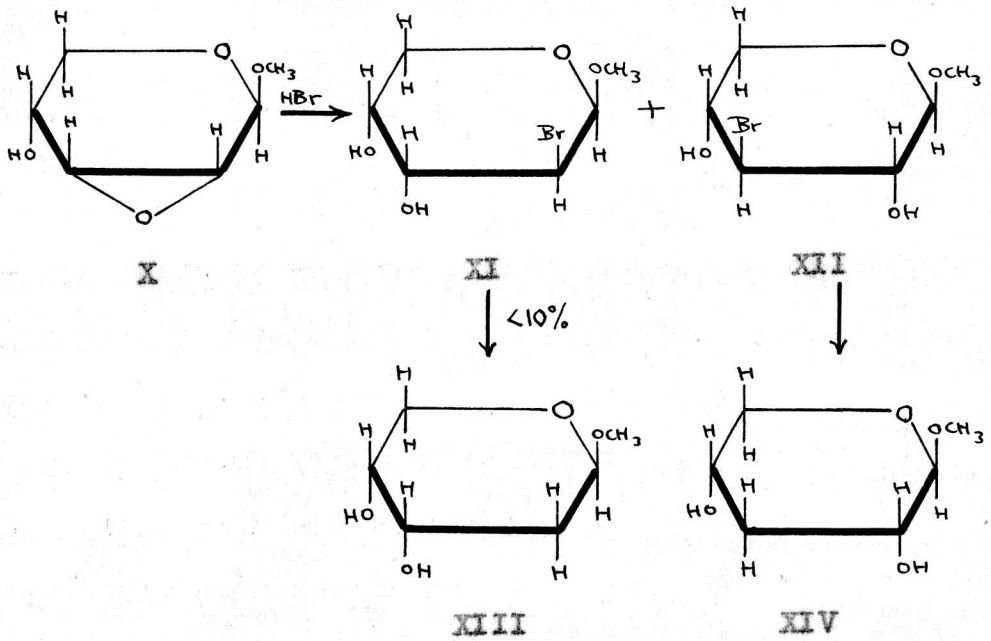


It is to be noted that while any one sugar will give two nitroalcohols when condensed with nitromethane, these in turn will give only one acetylated nitro-olefin since the formation of the double bond destroys the asymmetry of C_2 of the nitroalcohols. Overend et al.(81) repeated this synthesis using D-erythrose as starting material and obtained a very poor yield of 2-deoxy-D-ribose as end-product. This procedure does not appear to have been exploited further.

Another possibility was examined by Overend and Stacey(82): it is known that, in certain cases, methanesulphonyl residues attached to a secondary carbon atom may be replaced by an iodine atom by heating the methanesulphonate with sodium iodide in acetone(83). If such a reaction were possible with a 2-methanesulphonyl derivative of D-arabinose, a 2-iodo-derivative would be obtained and on reduction would lead to the formation of 2-deoxy-D-ribose. All attempts, however, to effect the exchange reaction between various 2-methanesulphonyl derivatives of D-arabinose and sodium iodide were unsuccessful.

The most fruitful, and, therefore, most widely employed methods for the synthesis of deoxy-sugars at the present time are those using ethylene oxide anhydro-sugars. Kent, Stacey and Wiggins(84) found that cleavage of the ethylene oxide ring in methyl

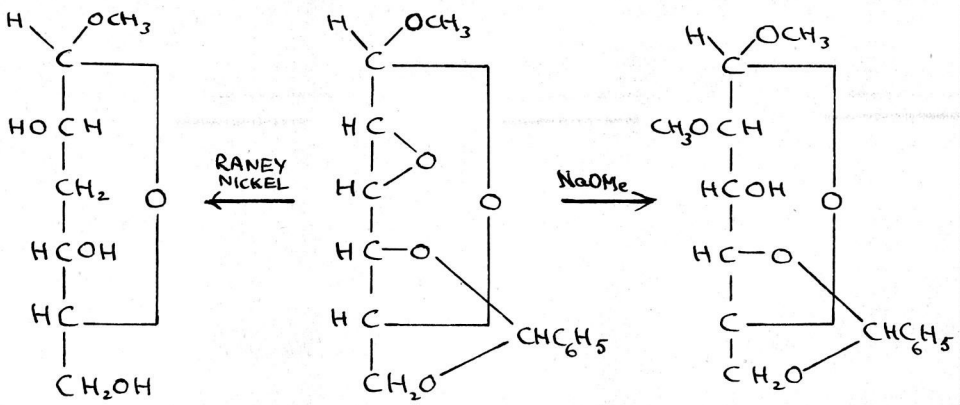
2:3-anhydro-β-D-riboside(X) with hydrogen bromide yielded mainly methyl 3-bromo-3-deoxy-β-D-xyloside (XII) together with less than 10% of methyl 2-bromo-2-deoxy-β-D-arabinoside(XI). The latter was then converted by reduction with Raney nickel into methyl 2-deoxy-β-D-riboside (-arabinoside)(XIII) and the former by the same treatment gave methyl 3-deoxy-β-D-riboside (-xyloside) (XIV). The yield of (XIII) was too small to be of practical value.



X XI XII XIII XIV

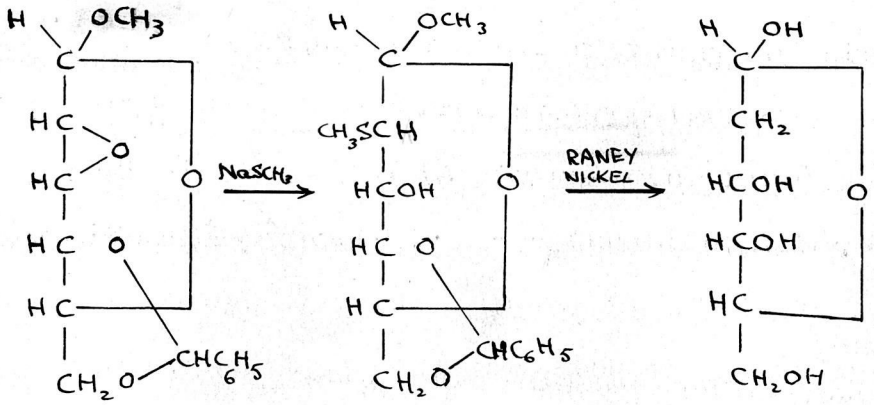
Methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside (XV) had become easily available through the work of Robertson and Griffith(54) and of Richtmyer and Hudson (85) and D.A. Prins(86) used this derivative to prepare the 3-deoxy-sugar. Robertson and Griffith had found that alkaline hydrolysis of this anhydro-compound with sodium methoxide caused cleavage of the oxygen bond nearer the glycosidic group and gave mainly methyl 2-methyl-4:6-benzylidene-α-D-altroside(XVI).

whereas Prins on reduction with Raney nickel found that fission occurred at the other oxygen bridge (the one further from the glycosidic group) and he obtained methyl 3-deoxy-α-D-glucoside(XVII)(-alloside).



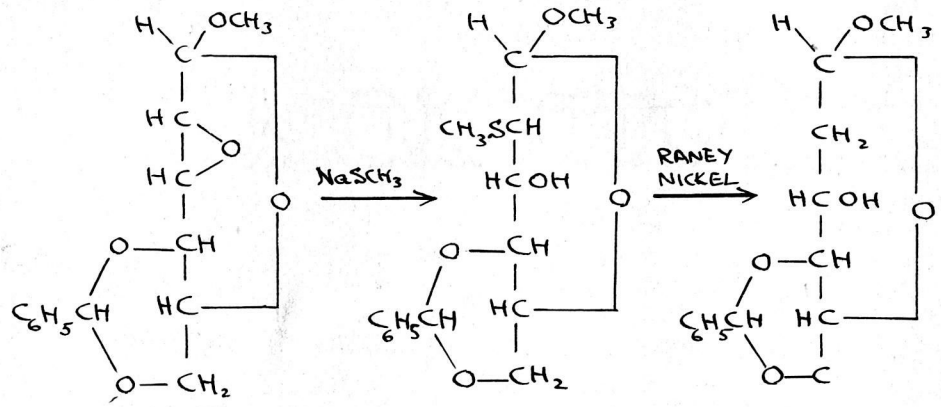
XVII XV XVI

Bougault and co-workers(87) and then Mozingo, Wolf, Harris and Folkers(88) found that certain sulphur-containing compounds, when submitted to hydrogenolysis with Raney nickel catalyst, undergo cleavage and the sulphur atom is replaced by two hydrogen atoms. Jeanloz, Prins and Reichstein(89) made use of this reaction to synthesise 2-deoxy-sugars. By treatment of methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside(XV) with sodium mercaptide a 2-methylthio-derivative(XVIII) was obtained, from which by Mozingo's reductive desulphurisation procedure the methyl 2-deoxy-α-D-alloside(XIX) was derived.



XV XVIII XIX

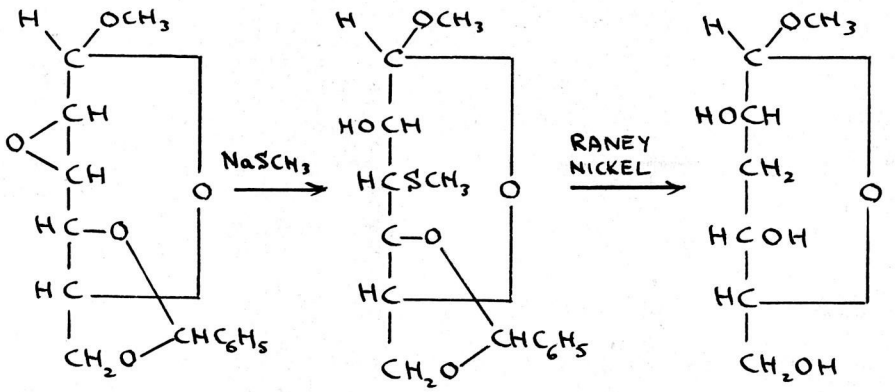
Further support for these results was obtained by Maehly and Reichstein(90) who achieved the isolat-:ion of the 2-deoxy-sugar(XXI) following similar treatment of methyl 2:3-anhydro-4:6-benzylidene- ∝-D-guloside(XX).



XX XXI

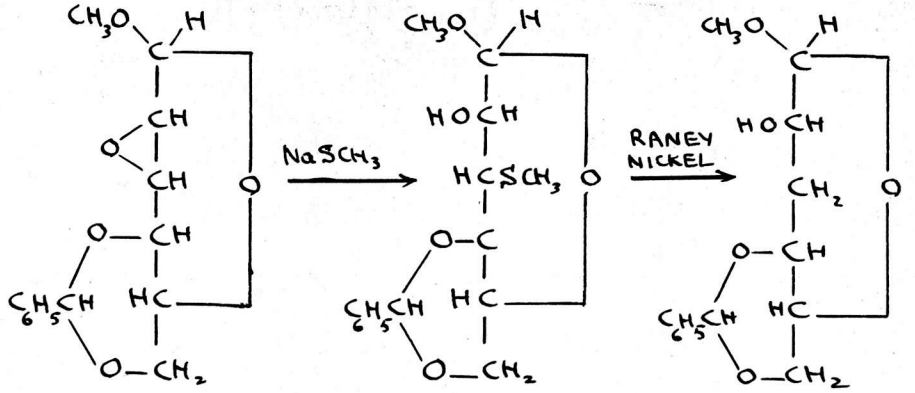
Subsequent work by Bolliger and Prins(91) showed however that 3-deoxy-derivatives might also be obtain-:ed by this method. They found that treatment of methyl 2:3-anhydro-4:6-benzylidene-∝ -D-mannoside(XXII) with sodium mercaptide gave a quantitative yield of

methyl 4:6-benzylidene-3-methylthio-β-D-altroside (XXIII) which on hydrogenolysis with Raney nickel gave the 3-deoxy-derivative (XXIV).



XXII XXIII XXIV

Similar treatment(92) of methyl 2:3-anhydro-4:6-benzylidene-β-D-taloside (XXV) also gave a 3-methylthio-derivative (XXVI) and a 3-deoxy-sugar (XXVII)



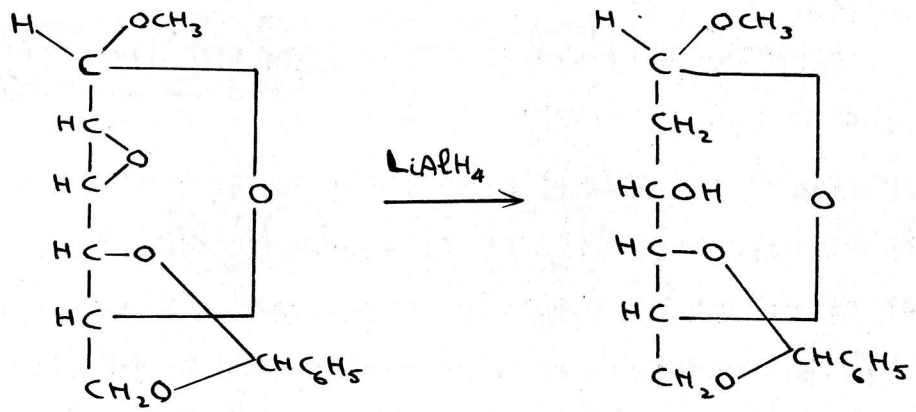
XXV XXVI XXVII

These results may be summarised as follows. Hydrogenation under pressure of methyl 2:3-anhydro-

4:6-benzylidene-α-D-hexosides with Raney nickel in methanol always gives methyl 3-deoxy-α-D-hexosides, regardless of the spatial configuration. On the other hand, the same anhydro-α-D-sugars (together with the β-form recorded above) afford on treatment with sodium mercaptide either 2- or 3- methylthio-ethers, according to the spatial arrangement. Here the scission of the ethylene oxide ring follows the same route as when sodium methoxide is the hydrolytic agent(p.17,20). Gut, Prins and Reichstein(92) advanced the following generalisation to cover these facts: In the D-series, the -SMe or -OMe group enters in the 2-position when the ethylene oxide ring is situated to the right in the Fischer projection formula of the sugar, and in the 3-position if the ring is to the left. The opposite will hold in the L-series: if the ethylene oxide ring is on the left, the substituent enters in the 2-position; if the ring is on the right, then the -SMe or -OMe group attaches itself to C_3. In comparing the theories advanced to account for the entry of the substituent (-SMe or -OMe group) in a particular position, the choice by Reichstein et al. of the Fischer projection formulae to illustrate their generalisation appears unfortunate since no structural significance attaches to whether the ethylene oxide ring is situated to the left or to the right of the sugar chain. The deciding factor

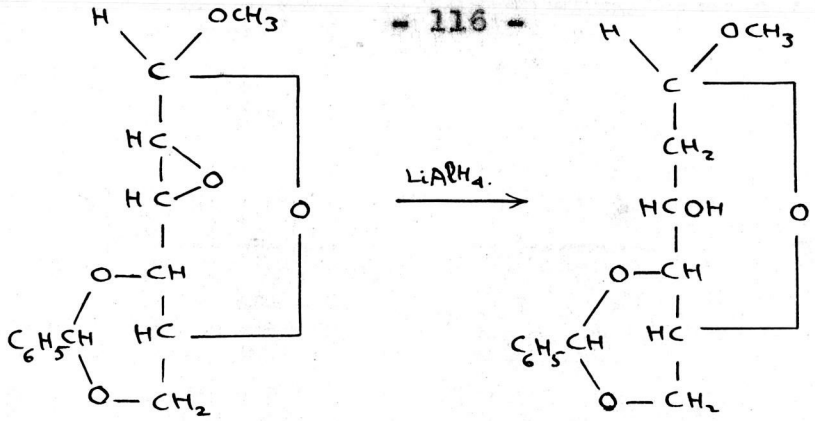
is rather the position of the anhydro ring relative to the glycosidic group and the terminal carbon atom as can be seen by the examination of a model.

In the most recent work on the synthesis of deoxy-sugars lithium aluminium hydride has been employed as the cleavage reagent of the ethylene oxide ring. D.A. Prins(93) using the general methods of Nystrom and Brown(94) succeeded in obtaining satisfactory yields of deoxy-sugars with this reagent. Methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside(XV) gave methyl 4:6-benzylidene-2-deoxy-α-D-alloside(-altroside) (XXVIII) (94).



XV XXVIII

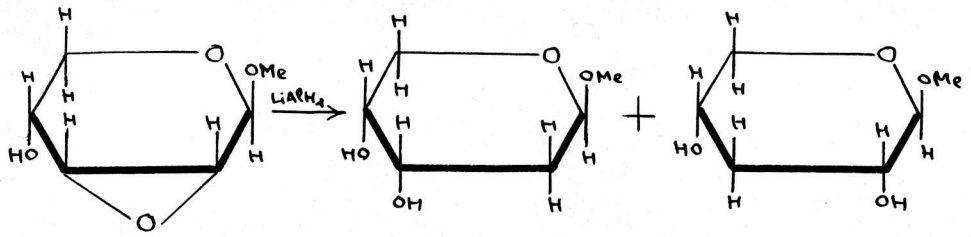
In the same way Hauenstein and Reichstein(95) converted methyl 2:3-anhydro-4:6-benzylidene-α-D-guloside(XX) into methyl 4:6-benzylidene-2-deoxy-α-D-guloside(XXI)(-idoside).



XX XXI

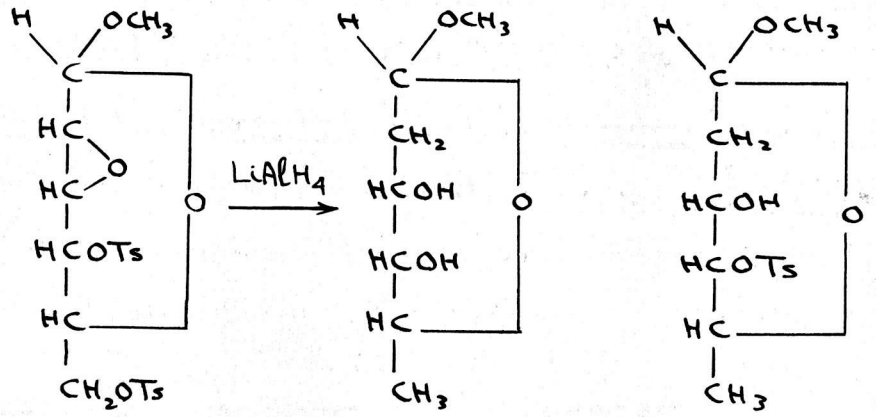
Bose, Chaudhuri and Bhattacharyya(104) cite the production of deoxyaltrose and deoxyidose derivatives from the reduction of 2:3-anhydro sugars with lithium aluminium hydride as further support for Fürst and Plattner's rule (p. 23) of polar substitution of the entering groups.

Allerton and Overend(96) succeeded in isolating both possible cleavage products from methyl 2:3-anhydro-β-D-riboside(X) with this reagent. The main product was methyl 3-deoxy-β-D-xyloside (-riboside) (XIV) together with approximately 14% of methyl 2-deoxy-β-D-riboside(-arabinoside)(XIII).



X XIII XIV

By varying the conditions of the lithium aluminium hydride reduction of methyl 2:3-anhydro-4:6-di-toluene-p-sulphonyl-α-D-alloside(XXIX), Bolliger and Ulrich(97) found that either methyl 2:6-dideoxy-α-D-alloside (methyl α-D-digitoxoside)(XXX) or its 4-toluene-p-sulphonyl derivative(XXXI) resulted.

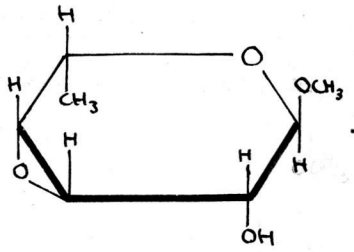


XXIX XXX XXXI

Further work by Bolliger and Thürkauf(98) showed that in this reaction, of the three functional groups in (XXIX) the anhydro ring reacts more readily than the primary toluene-p-sulphonyl group which, in turn, is more reactive than the secondary toluene-p-sulphonyl group.

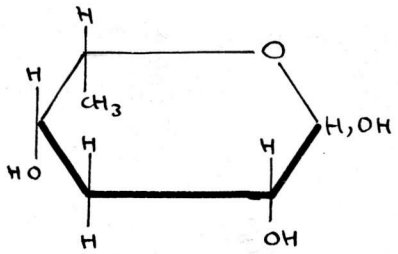
In the present work the action of lithium aluminium hydride on 3:4- and 2:3-anhydro-derivatives from 6-deoxy-L-mannose and 6-deoxy-L-galactose, respectively, has been investigated.

SYNTHESIS OF DIDEOXY-DERIVATIVES FROM L-RHAMNOSE.

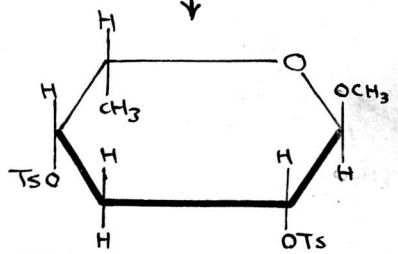


Methyl 3:4-Anhydro-6-deoxy-α-L-taloside.

$LiAlH_4$; H_2SO_4.

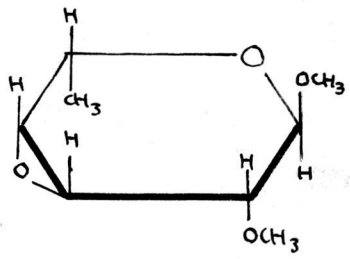


3:6-Dideoxy-L-talose.

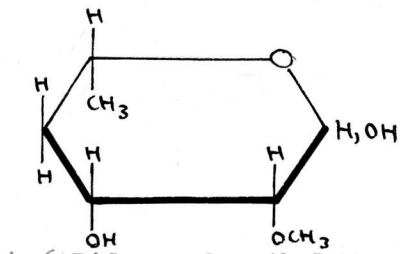


Methyl 3:6-Dideoxy-2:4-ditoluene-p-sulphonyl-L-talos**i**de.

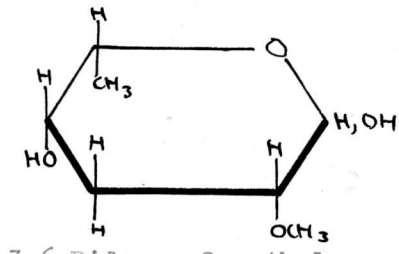
CH_3I Ag_2O



Methyl 3:4-Anhydro-6-deoxy-2-methyl-α-L-taloside.



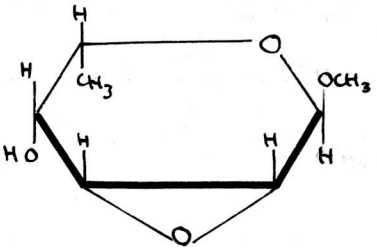
4:6-Dideoxy-2-methyl-L-talose.



3:6-Dideoxy-2-methyl-L-talose.

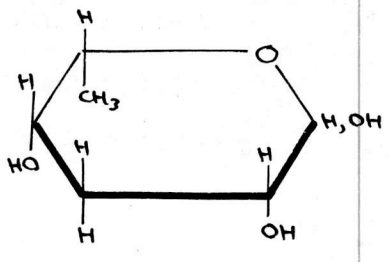
(only product actually obtained).

SYNTHESIS OF DIDEOXY DERIVATIVES FROM L-FUCOSE.

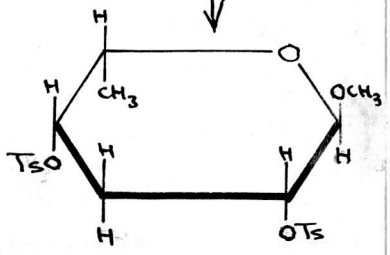


Methyl 2:3-anhydro-6-deoxy-α-L-taloside.

$LiAlH_4$, H_2SO_4.

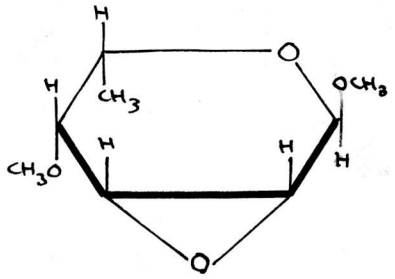


3:6-Dideoxy-L-talose.

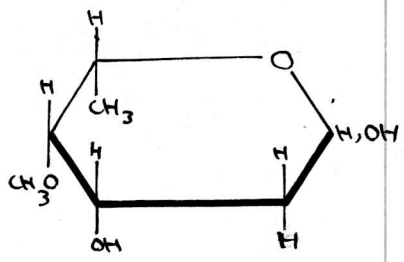


Methyl 3:6-Dideoxy-2:4-ditoluene-p-sulphonyl-α-L-taloside.

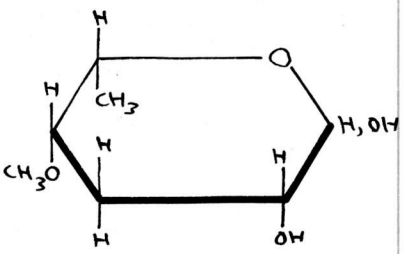
CH_3I Ag_2O



Methyl 2:3-anhydro-6-deoxy-4-methyl-α-L-taloside.



2:6-Dideoxy-4-methyl-L-talose.



3:6-Dideoxy-4-methyl-L-talose.

Derivatives from L-Rhamnose and L-Fucose.

EXPERIMENTAL

Reduction with Lithium Aluminium Hydride of:

(1) Methyl 3:4-anhydro-6-deoxy-∝-L-taloside.

Crystalline methyl 3:4-anhydro-6-deoxy-∝-L-taloside(2.0g.) in dry ether (150c.c.) was added dropwise to a suspension of finely powdered lithium aluminium hydride(2.0g.) in dry ether(150c.c.) contained in a three-necked flask. The suspension of the reducing agent was kept gently refluxing and well-stirred; the rate of addition of the ethereal solution of the anhydrotaloside was regulated according to the vigour of the reaction: after the initial brisk reaction had subsided, the solution of the anhydro compound was added more quickly but so that the addition required 30-45 minutes. The mixture in the reaction flask was then refluxed with continuous stirring for 4 hours. At the end of this period the flask was cooled in ice-water and the excess lithium aluminium hydride in the mixture destroyed by the careful addition of water. The strongly alkaline solution was then acidified (to litmus) with aqueous 2N. sulphuric acid and the whole mixture exhaustively extracted with cold chloroform (10 x 100c.c.). The dried (sodium sulphate) extracts, after removal of the solvent, afforded a pale yellow syrup which was purified by dissolution in water and treatment with "Hyflo" filtercel. The resultant colourless syrup

(0.7g.), $[\alpha]_D^{17°}$ -86°(c, 1.1 in water) was hydrolysed with aqueous sulphuric acid as required.

A portion of the above syrup(0.11g.) was hydrolysed at 100° with aqueous sulphuric acid(15c.c.; 4%) ($[\alpha]_D^{16°}$ -86° → -20°, 20 min. constant). The product, a colourless syrup, had $[\alpha]_D^{16°}$ -20°(c, 1.8 in methanol), -16°(c, 0.73 in water) and produced a single spot, R_G 0.72, on the paper chromatogram with solvent (I) as the eluant. On repetitions of this work, spotting of the hydrolysed product showed also faint traces of a second constituent, R_G 0.40, identical with L-rhamnose.

A portion (35mg.) of the hydrolysed product was treated with aniline as in previous experiments, but all attempts to isolate a crystalline anilide were unsuccessful.

Osazone Formation.

A portion (0.1g.) of the hydrolysed product was converted into the phenylosazone by treatment with phenylhydrazine(0.15c.c.) in water(2c.c.) and glacial acetic acid (1 drop) for 2 hours at 100°. An oil which failed to crystallise was isolated. The experiment was repeated in an atmosphere of carbon dioxide. After repeated washing and recrystallisation from aqueous ethanol, an amorphous solid was obtained which melted below 80°. All attempts to raise the m.p. were unsuccessful.

Synthesis of methyl 3:6-dideoxy-2:4-ditoluene-p-sulphonyl-α-L-taloside.

A portion (0.3g.) of the glycoside in dry, pure pyridine(25c.c.) containing drierite(10g.), after the slow addition of finely powdered toluene-p-sulphonyl chloride(1.05g.; 3 moles) was allowed to stand at 15° for 72 hours. At the end of this period, the dark red solution was poured with stirring onto crushed ice, when a small quantity of white solid was precipitated. This was removed by filtration; the filtrate was extracted with chloroform (4 x 50c.c.), the extracts washed with aqueous sulphuric acid(1:1; 3 x 50c.c.) followed by saturatedaqueous sodium hydrogen carbonate (2 x 50c.c.) and by water(50c.c.). The washed chloroform extracts were dried over anhydrous sodium sulphate and taken to dryness; a small quantity of syrup resulted which was poured onto crushed ice affording a further small quantity of white solid. The two solids were recrystallised from warm methanol and had m.p. 95°; they both gave a negative test for Cl^-.

(Found: S, 13.1; $C_{21}H_{26}O_8S_2$ requires S, 13.6%).

(2) Methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside.

Methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside (2g.) in dry ether(150c.c.) was added to lithium aluminium hydride(2g.) suspended in dry ether(150c.c.) as described above. The chloroform extracts of the worked-up mixture yielded on evaporation of the

solvent a colourless syrup(0.9g.) which had $[\alpha]_D^{16°}$ -83.3°(c, 1.4 in methanol). This (0.1g.) was hydrolysed with aqueous sulphuric acid(20c.c.; 4%) and the product, which had $[\alpha]_D^{16°}$-12.2°(c, 0.9 in methanol), produced on the paper chromatogram two spots, R_G 0.90 and R_G 0.72 (trace). The residue of the syrup(0.8g.) was then hydrolysed as above and the sugars separated by passage through a cellulose column, fractionation being achieved as on p. 38; the following fractions were collected.

Tubes.	
1 - 24	-
25 - 38	R_G 0.90
39 - 43	-
44 - 60	R_G 0.72

Identification of Fraction R_G 0.90.

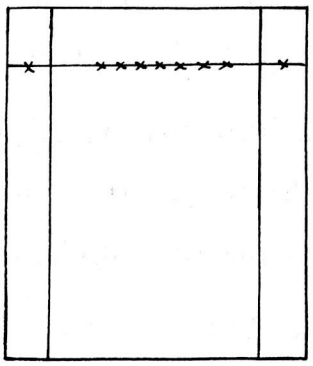
This fraction, a colourless syrup (0.305g. after purification) had $[\alpha]_D^{16°}$-14°(c, 3.05 in ethanol), -4° (c, 3.0 in water). (Found: OMe, 19.2; $C_7H_{14}O_4$ requires OMe, 19.14%). Demethylation of 45mg. with hydriodic acid in the usual manner gave a syrup which on heavy spotting produced on the paper chromatogram a single spot, R_G 0.72, indistinguishable from that given by 3:6-dideoxy-L-talose.

(3) Methyl 2:3-anhydro-6-deoxy-α-L-taloside.

Methyl 2:3-anhydro-6-deoxy-α-L-taloside(0.5g.)

in dry ether(30c.c.) was reduced with a suspension of lithium aluminium hydride(0.5g.) in dry ether(30c.c.) as already detailed. Removal of the solvent from the dried chloroform extracts of the acid aqueous layer gave a clear, pale yellow syrup (0.15g.). Chromatographic analysis with 8% phosphoric acid-aniline oxalate spray revealed one strong spot, R_G 1.0, and two faint spots, R_G 0.69 and R_G 0.30. Hydrolysis with aqueous sulphuric acid(20c.c.; 4%) at 100° (α_D -1.47 → -0.22, 60 min. constant), followed by neutralisation with barium carbonate, filtration and removal of water under reduced pressure gave a reducing syrup(0.11g.); this produced on the paper chromatogram, with solvent (I) as eluant, four spots, R_G 0.72, R_G 0.85, R_G 0.50 and R_G 0.22, the last three indicating traces only.

Since the quantity of syrup was too small to allow separation on a cellulose column, it (0.1g.) was separated into its constituents on Whatman No.3 filter paper, the procedure being as follows. The bulk of the syrup was spotted on the central part of the origin line of the paper, while two spots on strips 5cm. wide and situated on either side of the central portion, served for reference.



After the mobile phase (solvent (I)) had advanced to within one inch of the bottom of the chromatogram (20 hours), the paper was dried. The two 5cm. strips were then cut off and developed in the usual manner. Having determined the extent of movement of the various fractions on the reference strips, lines were drawn across the corresponding parts of the central portion of the paper to delineate the position of each sugar. These strips were then cut into small pieces and extracted with cold, aqueous ethanol (3 x 30cc.). After purification and removal of all solvents, the following chromatographically pure fractions were obtained:

(1) syrup(20mg.), R_G 0.22, indistinguishable from L-fucose.

(2) syrup(5mg.), R_G 0.50, indistinguishable from 6-deoxy-L-idose.

(3) syrup(50mg.), R_G 0.72, $[\alpha]_D^{16^\circ}$ -18°(c, 0.5 in methanol), -20°(c, 0.5 in water), indistinguishable from the sugar possessing similar rotations in water and methanol and producing a single spot of the same R_G 0.72, obtained by the reduction lithium aluminium hydride of methyl 3:4-anhydro--L-talosid e.

(4) syrup(10mg.), R_G 0.85. The quantity of this syrup was too small to allow further investigations to be made.

Repetition of this reduction and hydrolysis on a larger quantity(2.0g.) of methyl 2:3-anhydro-6-deoxy-α-L-taloside gave a product which on chromatographic analysis revealed a main constituent, R_G 0.72, accompanied by traces of substances the R_G values of which (R_G 0.22 and R_G 0.50) point to their being L-fucose and 6-deoxy-L-idose, respectively. No trace of the faster moving constituent, R_G 0.85, was obtained. The product(0.65g. after hydrolysis) was purified by passage through a column of cellulose and the following fractions were collected:

Tubes	1 - 130	-
	131 - 200	R_G 0.72
	201 - 300	unseparated mixture R_G 0.22 and 0.50

Identification of Fraction R_G 0.72.

This fraction, a colourless syrup (0.4g. after purification) had $[\alpha]_D^{16°}$ -20°(c, 1.2 in methanol). A portion (0.15g.) was converted into the glycoside by refluxing at 80° with methanolic hydrogen chloride (9c.c.; 1%) until the rotation became constant ($[\alpha]_D^{16°}$ -20° → -78°, 10 hours). The non-reducing product, a colourless syrup, was dissolved in dry pyridine (20c.c.) toluene-p-sulphonyl chloride (0.35g., 3 moles) added in the usual manner and the solution kept at 15° for 72 hours. On pouring onto crushed ice a sticky solid was obtained which, on repeated washing

with water, solidified and had after recrystallisation from warm methanol m.p. 94-95°.

(4) Methyl 2:3-anhydro-6-deoxy-4-methyl-α-L-taloside

Crystalline methyl 2:3-anhydro-6-deoxy-4-methyl-α-L-taloside(1.2g.) in dry ether(100c.c.) was reduced by lithium aluminium hydride(1.2g.) suspended in dry ether(100c.c.) according to the usual procedure. From the chloroform extracts a pale yellow syrup(0.65g.) was obtained and this was hydrolysed with aqueous sulphuric acid (40c.c.; 4%) at 100°(α_D -1.45 → -0.40, 30 min. constant). The hydrolysed product, a colourless reducing syrup (0.60g.) produced on the paper chromatogram, with solvent (I) as the eluant, one main elongated spot, R_G 0.93, accompanied by traces of three substances having R_G 0.72, R_G 0.50 and R_G 0.22. This product was purified by passage through a cellulose column but all attempts to separate the fraction R_G 0.93 into two components were unsuccessful. Fractionation was achieved as usual, every tenth tube being analysed chromatographically with solvent (I) as the eluant. The following fractions were collected:

Tubes	1-20	-
	21-35	R_G 0.93
	36-60	-
	61-65	R_G 0.72 presumed to be 3:6-dideoxytalose
	66-105	Unseparated mixture, R_G 0.50 and R_G 0.22

Identification of Fraction R_G 0.93.

This fraction(0.25g.) appeared to be contaminated with waxy material and after purification was reduced to 0.1g. It had $[\alpha]_D^{16°}$ -38°(c, 1.8 in water), -25° (c, 1.0 in ethanol). (Found OMe, 18.7; $C_7H_{14}O_4$ requires OMe, 19.14%). Careful spotting on a paper chromatogram and running for 48 hours gave what appeared to be two partly superimposed spots.

Demethylation with hydriodic acid in the usual manner afforded a syrup which on heavy spotting produced on the paper chromatogram two spots, R_G 0.63 and R_G 0.35, together with a trace of unchanged material. 3:6-Dideoxy-L-talose was run as a control and had R_G 0.71.

The syrup of R_G 0.93 gave a greenish-purple colouration in the Dische test.

Dische tests.- The procedure employed is that evolved by Deriaz et al.(125) as amended by Allerton, Overend and Stacey(124). The sugar was dissolved in water (concentrations recorded below) and the Dische reagent (4g. diphenylamine in 400cc. glacial acetic acid containing 11cc. concentrated sulphuric acid) (6cc.) added to it. The solution was heated in a standard test-tube in a boiling water-bath for 15 minutes; it was then cooled in ice-water for 10 minutes and the intensity of the colour developed measured in a "Unicam" photoelectric absorptiometer at λ 5800 Å. Furfuryl alcohol (0.02mg./cc.), which

gives in this reaction the standard blue colouration associated with 2-deoxy derivatives, was used for comparison. 3:6-Dideoxy-L-talose prepared from L-rhamnose and from L-fucose and 3:6-dideoxy-2-methyl-L-talose all gave a greenish-blue colouration with the Dische reagent, whilst the dideoxy-4-methyl-L-talose gave a greenish-purple colour. The following measurements were made:

	Optical density at 5800 Å	Molecular extinction coefficient ϵ
Furfuryl alcohol	0.45	2205
3:6-Dideoxy-L-talose (from rhamnose) / 3:6-Dideoxy-L-talose (from fucose)	0.40	112
3:6-Dideoxy-2-methyl-L-talose	0.35	110

The furfuryl alcohol gave in this test such an intense blue colouration that a concentration of 0.02g./litre had to be employed. The dideoxy sugars on the other hand required a concentration of 5-6g./litre for good development of colour. The following table summarises these observations.

Furfuryl alcohol		3:6-dideoxy-L-talose	
1.5mg./c.c.	v. dark	1.5mg./c.c.	hardly any colour
0.3mg./c.c.	dark blue	3 x 1.5mg./c.c.	" "
0.15mg./c.c.	dark blue	10 x 1.5mg./c.c.	v. pale green
0.02mg./c.c.	blue	5.4mg./c.c.	greenish-blue

It is to be noted that the colour given by furfuryl alcohol in this test developed within 2

minutes of heating, whilst the colourations produced by the dideoxy sugars required the full time of 15 minutes for development. It was also found that although the intensities of the colourations did not change for several hours, they deepened appreciably after 18 hours. The molecular extinction coefficient was calculated from the formula

$$\epsilon = \frac{\text{Reading x Molecular weight}}{\text{conc. in g./litre.}}$$

DISCUSSION.

The procedure employed for the reduction of ethylene oxide anhydrosugars with lithium aluminium hydride was broadly that of Nystrom and Brown(94) as applied by Lythgoe and Trippett(127). Various modifications have been introduced by Swiss workers in this field. Thus, Hauenstein and Reichstein(95) reflux the reaction mixture for a much shorter period (10 minutes) and add to the residue in chloroform a saturated solution of sodium potassium tartrate. In other cases, tetrahydrofuran is used as the solvent instead of ether, and ethyl acetate is employed in conjunction with aqueous Rochelle salt mixture(97). The conditions recommended by Lythgoe and Trippett were found to be satisfactory and were adopted throughout the present work.

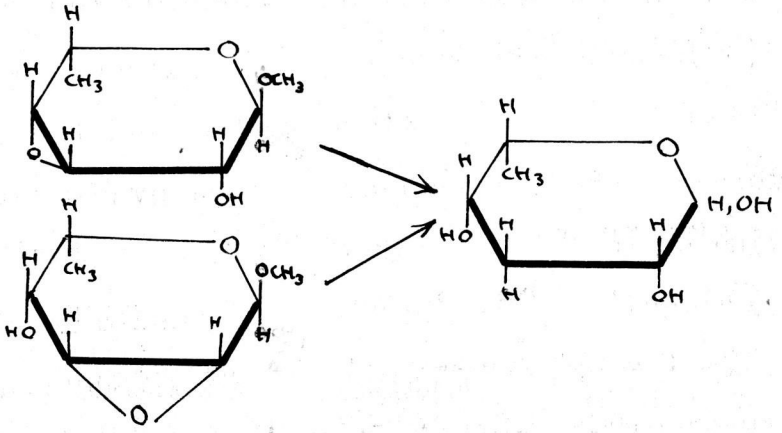
As all the anhydrosugars employed in this work were easily soluble in ether it was unnecessary to employ the Soxhlet extractor recommended for substances sparingly soluble in ether(94) or to experiment with different solvents such as tetrahydrofuran.

Following the procedure employed more recently by Chanda, Hirst and Percival(129), the whole of the reaction mixture was extracted with cold chloroform at the end of the reflux period, after the excess lithium aluminium hydride had been destroyed and the mixture acidified. In this way the reduced sugar was obtained in 40-45% yield, after hydrolysis with

aqueous sulphuric acid. The deoxy sugars were difficult to purify as they proved completely soluble in ethanol, chloroform and butanol-petrol ether, while their aqueous solutions remained turbid and could not easily be clarified.

In line with the findings of previous workers in this field, the reduction appears to afford one only of the two possible deoxy products. Thus Prins(93) obtained methyl 4:6-benzylidene-2-deoxy-α-D-alloside (-altroside) from methyl 2:3-anhydro-4:6-benzylidene-α-D-alloside; Hauenstein and Reichstein(95) converted methyl 2:3-anhydro-4:6-benzylidene-α-D-guloside into the 2-deoxy-α-D-guloside (-idoside) derivative; again, Bolliger and Ulrich(97) obtained methyl 2:6-dideoxy-α-D-alloside from methyl 2:3-anhydro-4:6-ditoluene-p-sulphonyl-α-D-alloside. Allerton and Overend(96) did succeed in isolating both methyl 3-deoxy-β-D-xyloside and methyl 2-deoxy-β-D-riboside (in 14% yield) from methyl 2:3-anhydro-β-D-riboside; in the majority of cases however, the reduction with lithium aluminium hydride appears to afford one only of the two possible cleavage products. In the present work, one only of the expected deoxy sugars was isolated in every case, albeit contaminated with small amounts of other sugars. Thus, from methyl 2:3-and methyl 3:4-anhydro-6-deoxy-α-L-taloside a single main product, R_G 0.72, was obtained after hydrolysis; this is presumed to be 3:6-dideoxy-L-

talose as this sugar is the only product that could result from the reduction of both the 2:3- and the 3:4-anhydrodeoxytaloside.



The products from both sources had similar optical rotations: $[\alpha]_D^{16°}$ -20°(c, 1.8 in methanol), -18°(c, 0.5 in methanol); $[\alpha]_D^{16°}$ -16°(c, 0.73 in water), -12°(c, 0.5 in water), respectively. They were indistinguishable chromatographically affording a single spot, R_G 0.72, with solvent (I) as the eluant, both alone and on admixture; their solutions had the same optical densities when these were measured in the course of a Dische test (see p.129); and they both afforded the same ditoluene-p-sulphonyl derivative. Attempts to isolate a crystalline anilide and phenylosazone were unsuccessful.

From methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside the main reduction product afforded, after hydrolysis and separation from contaminating traces, a single spot, R_G 0.90, on the paper chromatogram with solvent (I) as the eluant, and had $[\alpha]_D^{16°}$ -14°(c, 3.05 in ethanol), -4°(c, 3.0 in water). When submit-

ted to the Dische test this syrup gave a blue-green colour of similar intensity to that given by the 3:6-dideoxy derivative. Demethylation with hydriodic acid produced a syrup which on the paper chromatogram with solvent (I) as the eluant was indistinguishable from 3:6-dideoxy-L-talose. These results point to the reduction product being 3:6-dideoxy-2-methyl-L-talose.

Methyl 2:3-anhydro-6-deoxy-4-methyl-α-L-taloside after reduction and hydrolysis gave a syrup which appeared to contain two substances of almost identical R_G value. In the Dische test this product gave rise to a greenish-purple colouration in contrast with the greenish-blue colours obtained with the 3:6-dideoxy-taloses. The more obvious conclusion from these results is that the product is a mixture of 2:6-dideoxy- and 3:6-dideoxy-4-methyl-L-talose. If this were so, demethylation should give 2:6-dideoxy- and 3:6-dideoxy-L-talose. Unfortunately chromatographic analysis of the demethylation syrup, while revealing two substances, failed to show the presence of 3:6-dideoxy-L-talose. It is true that one of the spots has an R_G value close to that of 3:6-dideoxy-L-talose and the fact that it is slightly slower might be due to the hindering effect of the other substances present, but in the opinion of the writer this does not appear likely. The other constituent of the demethylation syrup, R_G 0.35, might well be the 2:6-dideoxy sugar, but this

substance has never been prepared and no constants are available for comparison.

Since chromatographic separation of the products obtained from the action of lithium aluminium hydride on methyl 2:3-anhydro-6-deoxy-4-methyl-α-L-taloside has proved impossible, a completely different line of approach will be necessary to establish the identity of these derivatives.

Deriaz et al.(125) submitted numerous carbohydrate derivatives to the Dische test(126) in an attempt to establish its specificity for 2-deoxyribose. They proved that the reaction depends upon the conversion of the 2-deoxypentose under acid conditions into ω-hydroxylaevulic aldehyde. This substance reacts with diphenylamine in the presence of glacial acetic acid and conc. sulphuric acid and gives rise to solid complexes some of which under acid conditions give a typical blue colouration and characteristic absorption band. These workers reached the conclusion that although certain carbohydrate derivatives produced colours under these conditions, the tests for 2-deoxy-ribose were not invalidated. Only those compounds such as 2-deoxy ribose, arabinal and furfuryl alcohol which are convertible into ω-hydroxylaevulic aldehyde gave colours of similar optical density when measured in a Spekker absorptiometer. Methyl 2-deoxy-dimethyl-β-L-ribopyranoside gave a much lower value for the molecular extinction coeff-

icient than the above substances, but this is to be expected as the transformation of this compound into ω-hydroxylaevulic aldehyde would be much more difficult inasmuch as it involves the cleavage of an ether methyl group.

This work was extended by workers in the Birmingham laboratories(128) to 2-deoxy-D-xylose which, on treatment with the Dische reagent, gave the characteristic blue colour. This again was shown to be due to the formation of ω-hydroxylaevulic aldehyde. Further work(124) on 3-deoxyxylose and 2:3-dideoxy-ribose showed that these substances also gave blue colours in the Dische test, but these colours were weak and not comparable with those obtained from the 2-deoxyderivatives and in these latter experiments it was often necessary to use higher concentrations of sugar and to heat for longer periods to obtain the colour.

The dideoxy derivatives obtained in this work gave results comparable with the above. It was necessary to use higher concentrations, to obtain the blue-green colour, than was used in the production of the standard colour from furfuryl alcohol; the values obtained for the molecular extinction coefficient for these derivatives appear to be reasonably close to those recorded for 2:3-dideoxy-L-ribose($\epsilon = 107$) and for 3-deoxy-L-xylose ($\epsilon = 126$), which is in agreement with their formulation as 3:6-dideoxy-hexoses.

SUMMARY.

Dideoxy derivatives from L-Rhamnose and L-Fucose.

1. Reduction of methyl 3:4-anhydro-6-deoxy-α-L-taloside with lithium aluminium hydride afforded, after acid hydrolysis, a single product shown to be 3:6-dideoxy-L-talose, as an identical product was obtained by the reduction of methyl 2:3-anhydro-6-deoxy-α-L-taloside. This reduction product gave a positive Dische test and was characterised by the isolation of crystalline methyl 3:6-dideoxy-2:4-ditoluene-p-sulphonyl-α-L-taloside.

2. Reduction of methyl 3:4-anhydro-6-deoxy-2-methyl-α-L-taloside afforded, after acid hydrolysis, 3:6-dideoxy-2-methyl-L-talose which gave a positive Dische test and was identified by demethylation to 3:6-dideoxy-L-talose.

3. Reduction of methyl 2:3-anhydro-6-deoxy-α-L-taloside, followed by acid hydrolysis, gave only 3:6-dideoxy-L-talose; this product had the same R_G value, the same rotation, and afforded the same ditoluene-p-sulphonyl derivative as the product from the reduction of methyl 3:4-anhydro-6-deoxy-α-L-taloside.

4. Reduction of methyl 2:3-anhydro-6-deoxy-4-methyl-α-L-taloside gave, after acid hydrolysis, a syrup which on chromatographic analysis revealed two substances of almost identical R_G value and which could not be separated.

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In conclusion, the writer would like to acknowledge his great debt to the late Dr. E.G.V. Percival, under whose expert and kindly supervision this research was begun; to express his gratitude to Professor E.L. Hirst, F.R.S., and to Dr. Elizabeth Percival for their greatly appreciated guidance and encouragement; and to thank Dr. C.A. Beevers for kindly taking the X-ray powder photographs, and the Seaweed Research Institute, Invereak, Midlothian for collecting the seaweed and supplying the L-fucose employed in Part II of this work.

Thanks are also due to the Distillers Company Limited for a maintenance grant.