SYNTHESES OF AMINO PENTOSES AND OF GLUCOSAMINE 6-PHOSPHATE

by

J. M. Anderson

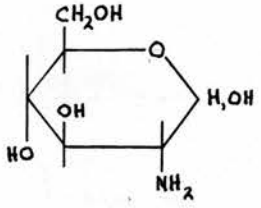
A thesis submitted for the degree of Doctor of Philosophy

Edinburgh September 1955

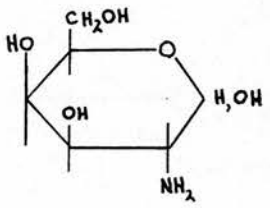
CONTENTS

INTRODUCTION

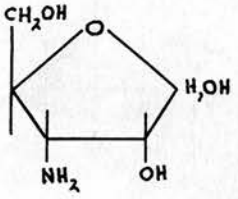
Only two naturally occurring amino hexoses have so far been isolated, glucosamine (2-amino-2-deoxy-glucose) and galactosamine (2-amino-2-deoxy-galactose). As the systematic name implies these substances are sugars in which a hydroxyl group is replaced by an amino group. Glucosamine and galactosamine are widely distributed in nature, as structural



D-Glucosamine



D-Galactosamine



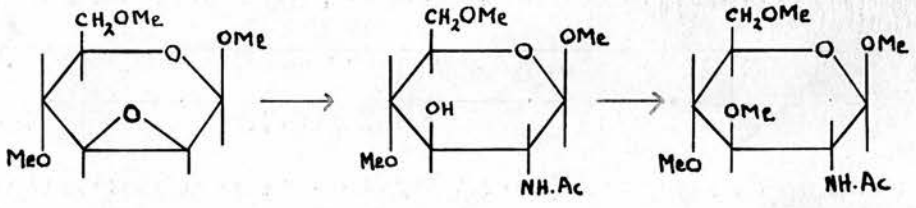
3-Amino-3-deoxy-D-ribose

components of the mucopolysaccharides[1]. These include such biologically important compounds as heparin, hyaluronic acid, tissue polysaccharides, and bacterial polysaccharides. Chitin, a polymer of N-acetyl-glucosamine, is present as the organic skeletal substance of insects, crustacea, and fungi. These amino sugars normally possess the D-configuration but the antibiotic streptomycin has been shown to contain N-methyl-L-glucosamine[2]. Recently an amino pentose (3-amino-3-deoxy-D-ribose) has been identified as a component of puromycin[3].

D-Glucosamine was first isolated by the hydrolysis of the chitin in lobster shells[4] and was shown to have a close structural relationship to D-glucose by its conversion to

D-glucosazone on treatment with phenylhydrazine.[5] The isolation of D-galactosamine was first reported from cartilage and tendon mucoproteins[6] and this amino hexose was shown by similar reasoning to be related to D-galactose.[7]

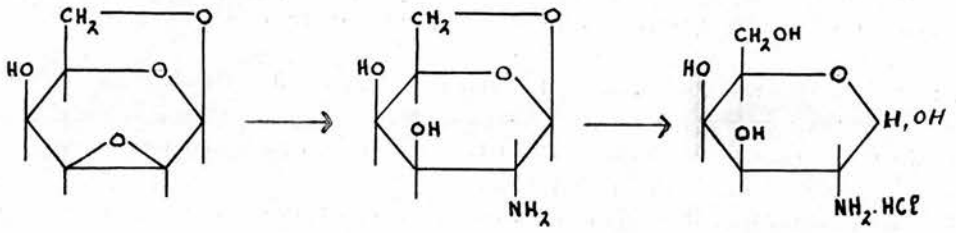
From 1912 to 1939, many efforts were made to establish the configuration of these two sugars. Much indirect evidence was obtained in the course of this work but no unequivocal proof of configuration was achieved until 1939 when Haworth, Lake and Peat[8] obtained definite evidence by synthetic methods. These authors treated methyl 4:6-dimethyl-2:3-anhydro-β-D-mannoside (I) with methanolic ammonia and obtained methyl 4:6-dimethyl-2-amino-2-deoxy-β-D-glucopyranoside isolated as its N-acetyl derivative (II). Methylation of this gave methyl 2-acetamido-2-deoxy-3,4,6-trimethyl-β-D-glucopyranoside (III), identical with the product prepared by methylation of the methyl N-acetyl-



(I) (II) (III)

β-D-glucosaminide obtained from naturally occurring glucosamine. In 1945[9] D-chondrosamine was synthesised by the action of ammonia on 1:6-2:3-dianhydro-β-D-talose (IV). 1:6-Anhydro-2-amino-2-deoxy-D-galactose (V) was obtained and this on hydrolysis with hydrochloric acid gave

2-amino-2-deoxy-D-galactose hydrochloride (VI) identical with naturally occurring D-galactosamine hydrochloride.

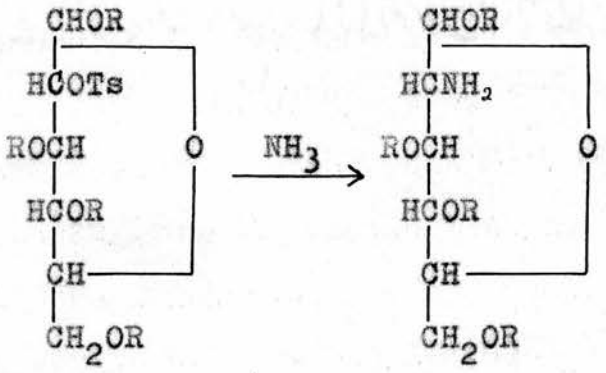


(IV) (V) (VI)

Foster and Stacey[10] have reviewed the chemistry of 2-amino sugars, the properties of which are similar in many respects to those of simple sugars. The active amino group, when unsubstituted and in its positively charged ionic form, exerts, however, a profound influence on the C_1 atom, with the result that a normally simple operation such as glycoside formation is very difficult by the ordinary methods. The inhibition of this acid catalysed reaction is presumed to be due to the repulsion of hydrogen ions by the positive charge on the neighbouring amino group[11]. This effect is evident also in the hydrolysis of the methyl glucosaminides. The hydrolysis constants for these reactions are some 100-200 times less than those of the methyl glucosides[11]. Nevertheless, when the amino group is blocked by a suitable substituent, glycoside formation can be more readily carried out. For instance, N-carbobenzyloxy-glucosamine readily gives the corresponding methyl glucosaminides on treatment with methanolic

hydrogen chloride[12]. However, the acetyl group appears to be exceptional in this respect for the methyl N-acetyl-glucosaminides can only be prepared with great difficulty from N-acetylglucosamine by treatment with methanolic toluene-p-sulphonic acid[13]. Instead it is necessary to treat a methanolic solution of this compound with an ethereal solution of diazomethane at low temperature to form the methyl glucosaminide[21]. The other hydroxyl groups in the molecule do not appear to be influenced by the amino residue.

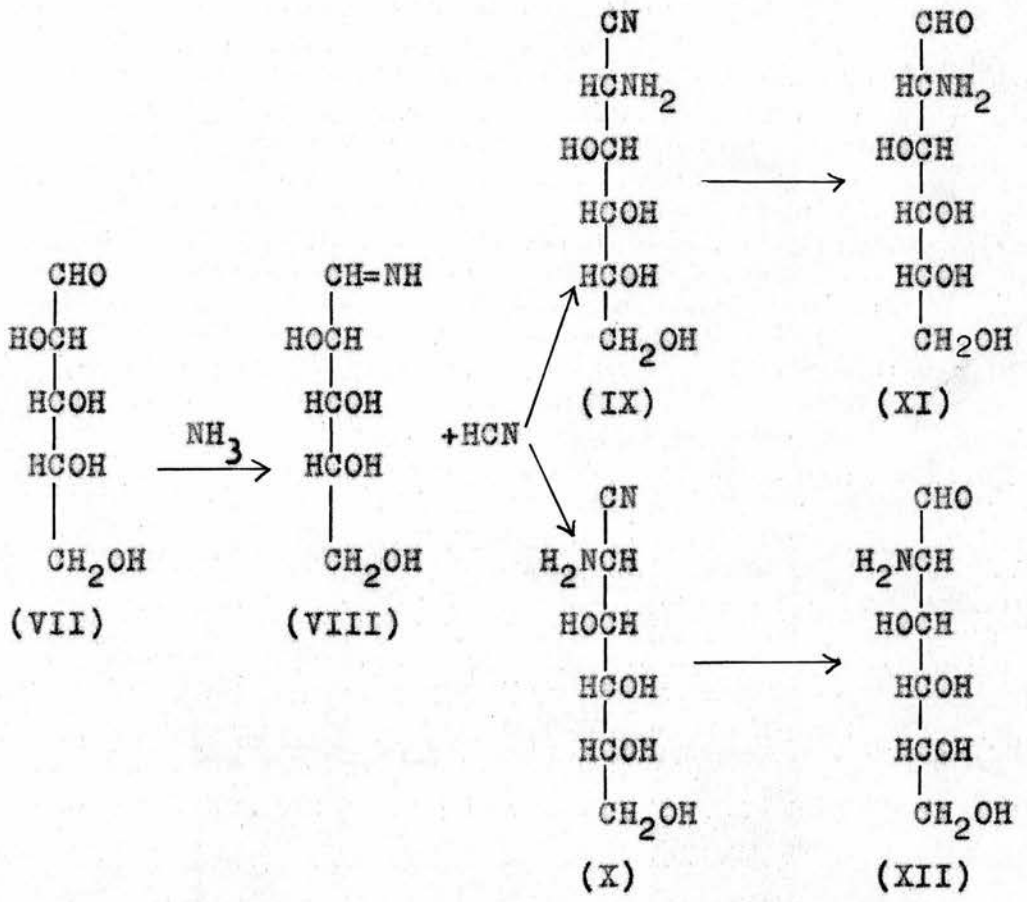
In recent years amino sugars have been synthesised by several methods. Treatment with ammonia of a toluene-p-sulphonyl sugar derivative in which all the remaining hydroxyl groups are protected by alkali-stable residues[14,22] leads to the direct replacement of the toluene-p-sulphonyloxy group by the amino residue, but the yields are invariably poor.



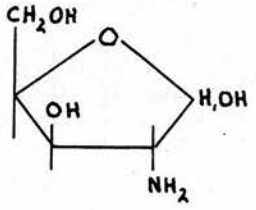
(R, an alkali-stable residue)

Another method is based on the glycosylamines (VIII) obtained by the action of ammonia on sugars (VII). The carbon chain is then lengthened by addition of hydrogen cyanide and two epimeric nitriles (IX) and (X) are produced, and these on hydrolysis and

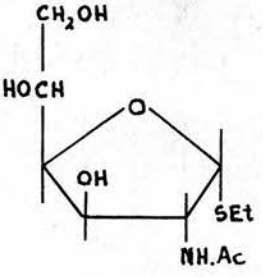
reduction give the corresponding amino sugars (XI) and (XII).[15]



A novel synthesis of 2-amino-xylose (XIII) has been reported by degradation, with sodium metaperiodate, of ethyl 2-acetamido-2-deoxy-α-D-glucothiofuranoside (XIV) followed by reduction and hydrolysis.[16]



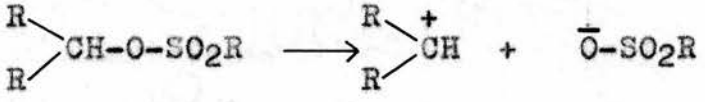
(XIII)



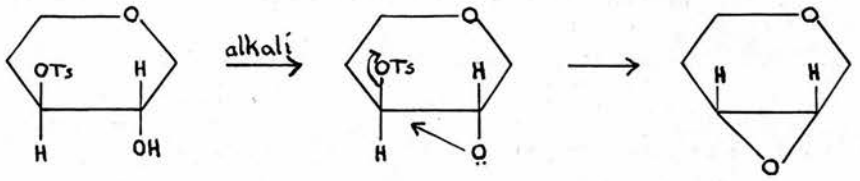
(XIV)

The usual method adopted for the synthesis of amino sugars involves the scission by ammonia of carbohydrate ethylene oxide ring compounds.

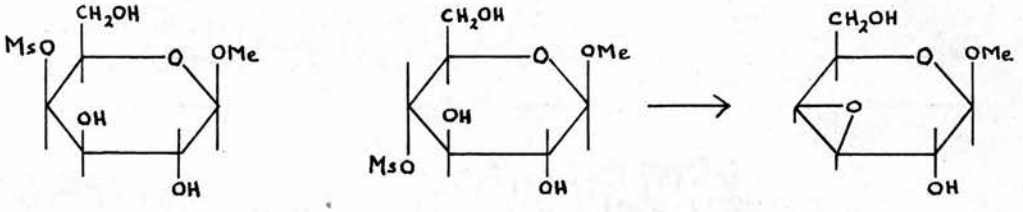
Anhydro sugars of various types have been studied for many years and a comprehensive review of the work carried out up to 1944 has been written by Peat.[17] It was not until 1928 that the first true example of the ethylene oxide type of anhydro sugar was synthesised. Since then many of these derivatives have been prepared, usually by the saponification of a sulphonic acid ester of a sugar. This reaction is recognised as following a fundamentally different course from the saponification of a carboxylic ester. Peat[17] considers that the hydrolysis of a sulphonic acid ester involves a break between the carbon atom of the chain and its attached oxygen atom with the formation of a carbonium cation.



This hydrolysis will occur readily only if there is a suitably situated nucleophylic group in the molecule. A hydroxyl group on an adjacent carbon atom, if trans situated with respect to the sulphonyloxy residue, satisfies this condition. The oxygen atom of the hydroxyl group displaces the sulphonyloxy group with the formation of an ethylene oxide ring. This involves a Walden inversion at the carbon atom previously occupied by the sulphonyloxy group.



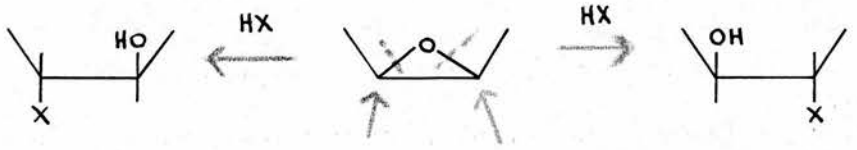
This necessity for trans relationship between the hydroxyl and sulphonyloxy groups is illustrated by comparison of the action of sodium methoxide on methyl 4-mesyl-β-D-galactopyranoside (XV) and methyl 4-mesyl-β-D-glucopyranoside (XVI).[18] The glucoside is rapidly transformed into methyl 3:4-anhydro-β-D-galactopyranoside (XVII), but sodium methoxide is without effect on the galactoside.



XV XVI XVII

The mode of scission of these oxide rings in sugars has in recent years aroused considerable interest. The reaction is intermolecular and the attacking ion becomes part of the final product. It is considered that either carbon atom involved in the oxide ring can develop cation activity, and that the attacking ion then approaches the active centre on the side opposite from that originally occupied by the oxide ring, union occurs and this results in a change of configuration on the carbon atom which acquires the attacking ion. In many of these

derivatives studied, both centres appear to become active and a mixture of two different sugars is produced on scission of the ring, both of which are configurationally different from the ring compound itself.



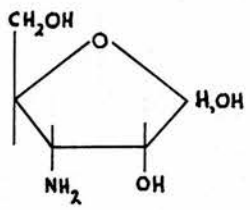
Reagents commonly used to study this scission include hydrochloric acid (giving C-chloro-derivatives), sodium hydroxide solution (giving free sugars), methanolic sodium methoxide (giving mono-O-methyl sugars), lithium aluminium hydride (giving deoxy sugars), and ammonia. The action of dry methanolic ammonia is regarded[17] as an exchange of the NH_2^- anion derived from ammonia as follows:

$$NH_3 \longrightarrow NH_2^- + H^+$$

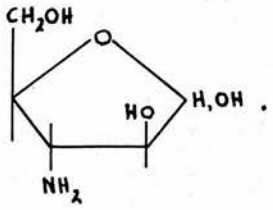
and the products are amino sugars.

These reactions have been studied by many investigators, generally as part of a study of the mode of scission of ethylene oxide rings, and several amino hexoses have been prepared from these ring compounds. More recently the method has been used for the preparation of amino pentoses. The antibiotic puromycin was shown[3] to contain 3-amino-3-deoxy-D-ribose (XVIII) and Baker and his collaborators[3,19] have

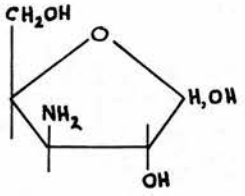
synthesised the methyl furanosides and pyranosides of this sugar. Derivatives of 3-amino-3-deoxy-D-arabinose (XIX) and 3-amino-3-deoxy-D-xylose (XX) were also prepared in the course of this work.



(XVIII)



(XIX)

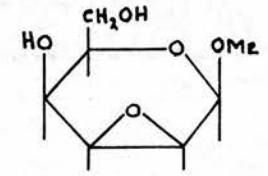


(XX)

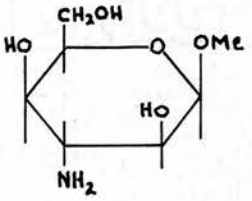
The separation of the two amino sugars produced on ammonolysis of the oxide ring has invariably proved difficult. Fractional crystallisation has proved successful in some experiments, and it has generally been found that one amino sugar is formed in greater quantity than the other. Although several hypotheses have been advanced, based on the conformation taken up by the pyranose sugar ring, to account for this preferential cleavage of the ethylene oxide ring,[20] it is not yet possible to predict, on scission of a particular ring compound, which of the two possible products will be formed in the larger amount.

The characterisation of the resulting amino sugars, unless they are derivatives of either glucosamine or galactosamine, has proved exceedingly difficult since very few authentic reference compounds of amino sugars have been prepared. When it has proved impossible to obtain authentic specimens of the possible products, then identification has been by analogy

with the products of scission of the ethylene oxide compound with sodium methoxide. It is assumed that the mechanism of scission of the ethylene oxide ring is the same with sodium methoxide (active ion OCH_3^-) as it is with ammonia (active ion NH_2^-) and that both show the same preference for one particular direction of ring opening. In other words it is assumed that a particular ethylene oxide derivative of a sugar, whether it is split by ammonia or sodium methoxide, gives rise preferentially to a derivative of the same sugar. For example Wiggins[22] identified the crystalline derivative obtained in 64% yield from the ammonolysis of methyl 2:3-anhydro-β-D-taloside (XXI) as methyl 3-amino-3-deoxy-β-D-idoside (XXII) by analogy with the 64% yield of methyl 3-methyl-4:6-benzylidene-β-D-idoside (XXIV) obtained on treatment of methyl 2:3-anhydro-4:6-benzylidene-β-D-taloside (XXIII) with sodium methoxide.

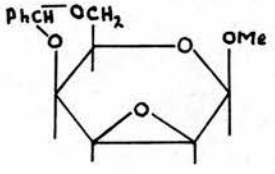


NH_3 →

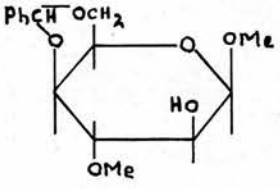


(Major product)

(XXI) (XXII)



NaOMe →



(Major product)

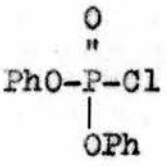
(XXIII) (XXIV)

The present investigation is concerned with the mode of scission of ethylene oxide rings in lyxofuranose derivatives. Here the sugar ring is considered to be planar and the conformation taken up by the sugar cannot be the deciding factor in the preferential cleavage of the oxide ring. At the same time new amino pentose sugars have been synthesised and characterised.

The phosphate esters of sugars are of great biological importance since they act as intermediates in the breakdown of glycogen to lactic acid in the muscle, in the fermentation of sugars to alcohol and other products, and, possibly, in oxidative metabolic processes in general. The specially important group of hexose phosphates includes glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-diphosphate. As a group, the phosphoric esters of sugars are strongly acidic and the materials have been isolated in many cases as the crystalline barium, calcium, lead, sodium, or alkaloid salts. Synthetic carbohydrate phosphoric esters were first obtained by the direct phosphorylation of the free sugars and the products from most experiments were mixtures containing phosphate groups attached to various carbon atoms in the sugar chain. The phosphorylating agent used was phosphorus oxychloride at low temperature in the presence of a neutralising agent such as pyridine. To prevent reaction with more than one hydroxyl group, it has often been necessary to block the other groups. Isopropylidene and acetyl groups have been used successfully in many experiments for this purpose by Levene

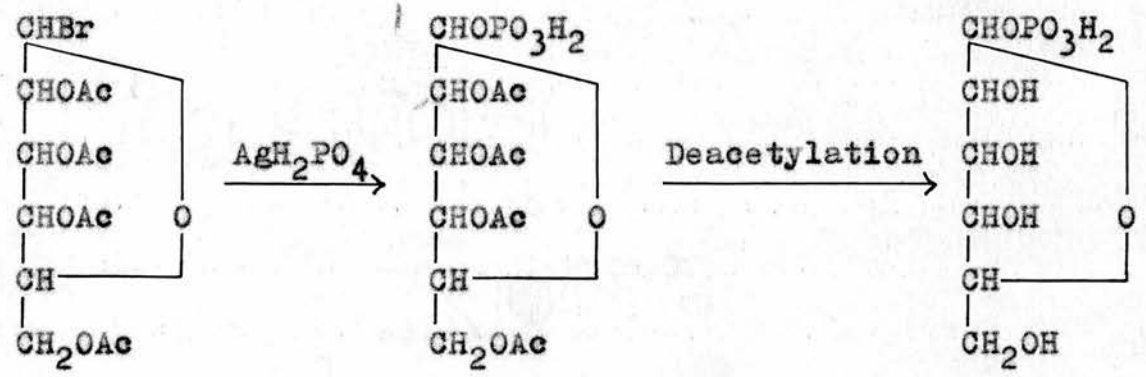
and his associates.[23]

More recently diphenyl phosphorochloridate (XXV) has been introduced as a phosphorylating agent[24] and has been extensively used.



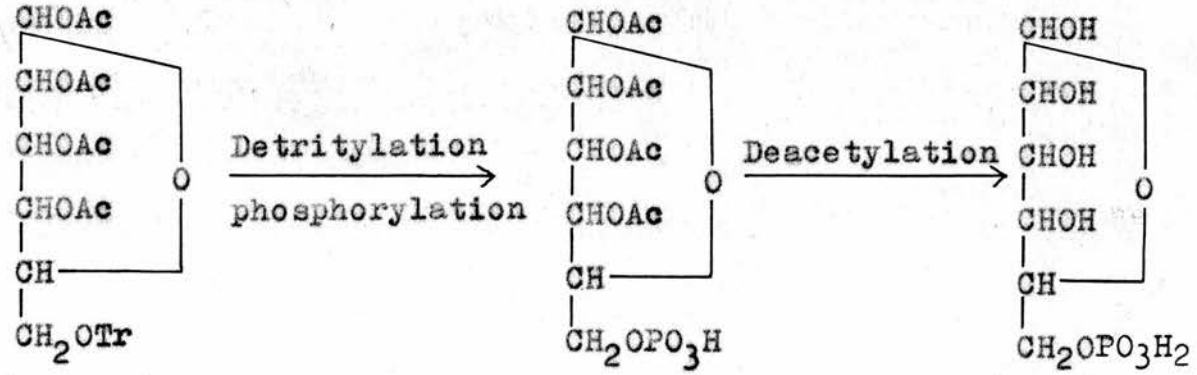
(XXV)

For phosphate esters substituted at the glycosidic carbon atom (XXVII) the usual method of preparation is the treatment



(XXVI) (XXVII)

of the tetra-acetyl-glycosyl bromide (XXVI) with silver phosphate followed by deacetylation.[25] For hexose 6-phosphates (XXIX), the usual method involves the preparation of the tetra-acetyl-6-trityl derivative (XXVIII) followed by



(XXVIII) (XXIX)

detritylation, phosphorylation and deacetylation.[26,27] Direct phosphorylation methods have recently been reported to give reasonable yields of glucose 6-phosphate directly from glucose.[28,29]

Harpur and Quastel[30] and Grant and Long[31] have reported that glucosamine is phosphorylated at the expense of adenosine triphosphate in the presence of aqueous extracts of ox brain which had been dried with acetone and powdered. The enzyme involved is presumably brain hexokinase. N-Acetyl-glucosamine which is not phosphorylated acts as a competitive inhibitor in this reaction. No attempt was made to isolate the glucosamine phosphate formed. Brown too has reported the rapid phosphorylation of glucosamine by A.T.P. in the presence of crystalline yeast hexokinase.[32] The product was isolated as the barium salt of an 'acid-stable' reducing phosphate ester of glucosamine. The constitution of this derivative was established, by elemental analysis and by the extent of oxidation by periodate, as glucosamine 6-phosphate. Brown[33] has also shown that this glucosamine 6-phosphate is converted to glucosamine 1-phosphate by phosphoglucomutase from rabbit muscle. The equilibrium mixture in this conversion contains about 20% of the 1-phosphate.

Not a great deal is known about the exact significance of glucosamine 6-phosphate in metabolic processes. Enzymes are found in Neurospora crassa which will convert hexose

6-phosphates to glucosamine 6-phosphate, acetylate the latter, and interconvert the acetyl-glucosamine 1- and 6-phosphates produced.(34) Brown(32) considers that the phosphorylation of glucosamine by hexokinase may be important in the series of anabolic stages whereby naturally occurring polysaccharides containing this sugar are produced. Kalckar and Klenow(35) suggest that the formation of glucosamine 6-phosphate should also be considered as a pathway in the biosynthesis of purine ribosides.

When the present work was begun no chemical synthesis of glucosamine 6-phosphate had been reported. In view of the markedly increased interest in this metabolite in recent years and the fact that the amounts prepared by biosynthetic methods have been very small, it appeared desirable to synthesise and characterise this compound by chemical means. Not only would it be of value as a reference compound but, if easily available, would enable studies to be made of its behaviour when treated with various enzyme systems. In this way some insight might be gained into the way in which glucosamine is utilised in nature.

PART I

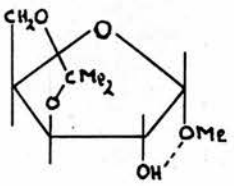
The Syntheses and Ammonolyses of Derivatives of 2:3-Anhydro-D-lyxose.

DISCUSSION

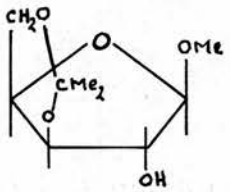
Amino sugars have been synthesised from xylose through the methyl 3:5-isopropylidene-2-toluene-p-sulphonyl-D-xylofuranosides and the 2:3-anhydro-sugars. In the course of this work 2:3-anhydro-D-lyxofuranoside and its 5-methyl ether have been prepared by the method of Percival and Zobrist.(67) the former in an overall yield of 32% from xylose. (Formulae of the intermediates prepared during the syntheses of the 2:3-anhydro-lyxose derivatives will be found on p. 30). The anhydro-sugar was isolated as the crystalline α-methyl furanoside and as a syrupy mixture of the two anomers. In 1955, Baker, Schaub and Williams(19)* reported the synthesis of both anomers of this derivative. While Percival and Zobrist used, as an intermediate, a syrupy mixture of the methyl 3:5-isopropylidene α- and β-D-xylofuranosides, Baker partly separated this material, by distillation, into its two anomers. This author considered that the separation was facilitated by the cis and trans position of the glycosidic methoxyl group with regard to the hydroxyl group on C_2. In the α-furanoside the methoxyl group which is cis to the hydroxyl group on C_2 is considered to be in a favourable position for intra-molecular hydrogen bonding and this is

* As this reference occurs frequently it is given henceforth in the abbreviated form Baker.(19)

thought to lower the boiling point, whereas in the β furanoside the glycosidic methoxyl is trans to the hydroxyl on C_2 and can therefore only bond intermolecularly in the normal fashion of alcohols.



Methyl 3:5-isopropylidene-α-D-xylofuranoside



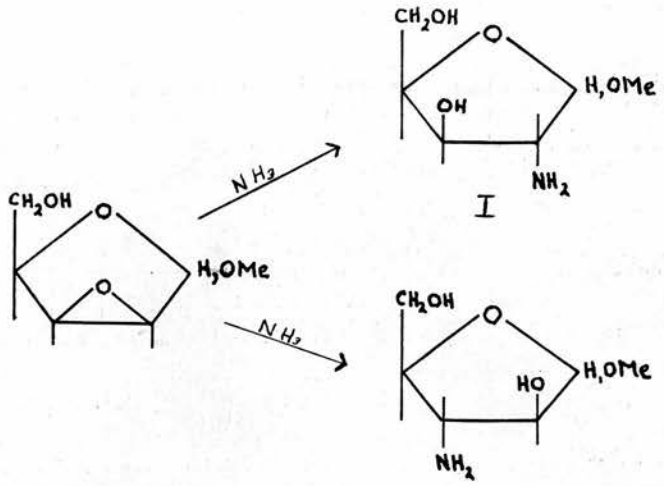
Methyl 3:5-isopropylidene-β-D-xylofuranoside

Although Baker carried out the subsequent syntheses on the two separate fractions and isolated crystalline α and β methyl furanosides of the anhydro-sugar none of his intermediate derivatives were obtained in a crystalline form. In the present work on the large scale preparation of the anhydro-sugar, separation by distillation of the anomers of the isopropylidene derivative was attempted. The specific rotations of the two fractions differed considerably from the values recorded by Baker.[19] The more volatile ('α') fraction had $[\alpha]_D$ $+75^\circ$ (Baker records $+18^\circ$) and the less volatile ('β') fraction had $[\alpha]_D$ -80° (Baker records -64°). That this was a more efficient separation was confirmed by the ready isolation of the two crystalline methyl 3:5-isopropylidene-2-toluene-p-sulphonyl-xylofuranosides. Hydrolysis of the isopropylidene group with boiling

1% methanolic hydrogen chloride, as used by Percival and Zobrist,[66] caused extensive anomerisation. Baker[19] claims that 80% acetic acid at 50° caused only 15% conversion. As this was shown to be unsatisfactory in the present work, 0·1% methanolic hydrogen chloride at room temperature for 30 minutes was used to hydrolyse this group. No anomerisation occurred and methyl 2-toluene-p-sulphonyl-α-D-xylofuranoside was obtained crystalline. During preliminary hydrolysis experiments with the β-anomer, a crystalline ditosyl ester of methyl β-D-xylofuranoside was isolated. Partial hydrolysis of the isopropylidene residue had presumably occurred during tosylation and the ditosyl compound had remained with the monotosyl isopropylidene derivative even after recrystallisation. No structure can be assigned definitely to this compound but if the hydroxyl groups in xylose and those in glucose[69] have the same relative reactivities, then it is probably methyl 2:5-di-toluene-p-sulphonyl-β-D-xylofuranoside.

Although methyl 2-toluene-p-sulphonyl-β-D-xylofuranoside could only be obtained as a syrup, good yields of both crystalline methyl 2:3-anhydro-D-lyxofuranosides were readily obtained on hydrolysis of the monotosyl esters.

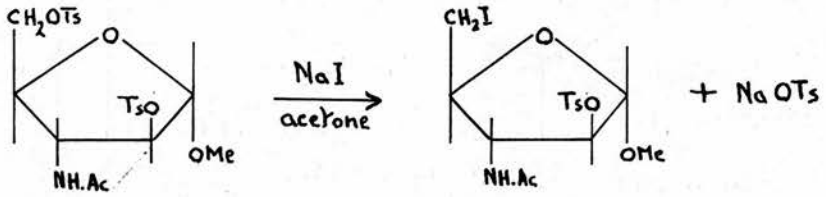
Two amino sugars, methyl 2-amino-2-deoxy-D-xylofuranoside (I) and methyl 3-amino-3-deoxy-D-arabofuranoside (II), can in theory be obtained on scission with ammonia of the ethylene oxide ring of methyl 2:3-anhydro-D-lyxofuranoside.



If the mechanism of the action of ammonia on the oxide ring is the same as that of sodium methoxide then, by analogy with the results of Percival and Zobrist,[67] the amino-arabinose derivative would be expected to predominate. Preliminary experiments on the ammonolysis of methyl 2:3-anhydro-α-D-lyxofuranoside led to the isolation of a crystalline N-acetyl derivative in 54% yield.* Since this compound failed to condense with acetone it was presumably not the 2-amino-xylofuranose derivative. The action of toluene-p-sulphonyl chloride at room temperature on the N-acetyl derivative showed that no change in the ring form from furanose to pyranose had occurred as the di-tosyl derivative isolated (III) gave a positive test for the presence of a primary toluene-p-sulphonyloxy group on

* Formulae of the amino-arabinose derivatives prepared from this ammonolysis and subsequent ammonolysis products may be found on pp. 45, 46.

treatment with sodium iodide in acetone.[63] Jeanloz[45]



III

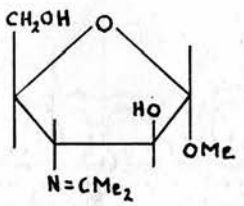
postulates the partial formation of an N-tosyl derivative of N-acetyl-glucosamine in a tosylation carried out at 50°. That this did not occur in the present work was indicated by parallel experiments carried out at room temperature on penta-acetyl-β-D-glucosamine, where no evidence for the formation of an N-tosyl derivative could be obtained. In addition Tipson[57] states that an N-tosyl group would not be replaced by iodine under the conditions used. This was borne out by the failure to isolate sodium tosylate on treatment of toluene-p-sulphonamide with sodium iodide in acetone.

That the crystalline N-acetyl derivative was methyl 3-acetamido-3-deoxy-α-D-arabofuranoside was further confirmed by hydrolysis with 3N hydrochloric acid at 100°. A crystalline β hydrochloride was isolated, the specific rotation of which differed from that recorded for the hydrochloride of 2-amino-2-deoxy-D-xylose.[16]

After the above work had been carried out, Baker[19] reported the isolation of the same crystalline N-acetyl derivative. This author identified it as the 3-amino-

arabinose compound because hydrolysis led to the isolation of a crystalline hydrochloride which gave a negative ninhydrin test in 1·5% sodium hydroxide solution. The constants obtained for the hydrochloride in the present work were in good agreement with those recorded by Baker for the same derivative.

When the ammonolysis was carried out on the large scale, attempts were made to obtain a more complete separation of the amino-arabinose derivative by solution of the crude ammonolysis product in hot acetone. This gave, on cooling, an 85% yield of the crystalline N-isopropylidene derivative (IV) prepared by Baker,[19] the specific rotation



IV

of which agreed with the value recorded by the latter author. N-Acetylation of this compound gave a good yield of methyl 3-acetamido-3-deoxy-α-D-arabofuranoside identical with that obtained in the preliminary experiments. Although readily crystalline, no sample of the N-isopropylidene derivative could be obtained which gave a sharp melting point. Three recrystallisations or drying at 100°/15 mm. for 8 hours brought about little improvement. Elemental analysis also indicated contamination. The

extreme sensitivity of the isopropylidene group to acid suggests that the contaminant may be the hydrolysis product, methyl 3-amino-3-deoxy-α-D-arabofuranoside, and the analytical figures are in agreement with this. Baker[19] records a 54% yield of the N-isopropylidene-amino-arabinose from the ammonolysis of the crude anhydro-sugar and a 90% yield from an 'analytically pure' sample.

After removal of the α N-isopropylidene 3-amino-arabinose derivative, the residual material (10% of theoretical) from the mother liquors was investigated for the presence of xylose derivatives. It was hoped to isolate a crystalline methyl N-acetyl-3:5-isopropylidene-xyloside but N-acetylation gave a syrup which failed to yield a crystalline derivative on condensation with acetone. Separation of any isopropylidene derivative was attempted by fractionation of the resulting syrup between chloroform and water. The chloroform fraction gave a very dark brown non-reducing syrup in an overall yield of 0·2% from the crude ammonolysis product. The non-reducing dark brown syrup (5% yield from the crude ammonolysis product) obtained on evaporation of the water soluble fraction showed, on chromatographic examination the presence of two major components having R_G values close to, but not identical with, that of methyl 3-acetamido-3-deoxy-α-D-arabofuranoside. One of these components may be the corresponding 2-acetamido-2-deoxy-xylose derivative but no crystalline compound could be isolated.

From the ammonolysis of the 'syrupy' methyl 2:3-anhydro-αβ-D-lyxofuranoside, after N-acetylation, 21% of a crystalline product was isolated. This was proved, as before, to be a methyl 3-acetamido-3-deoxy-D-arabofuranoside, but a mixed m.p. determination and specific rotation measurement showed it to be different from the α form already obtained. The constants for this compound were, however, in good agreement with those subsequently reported for the β-anomer by Baker.[19] It is worthy of note that the large difference in the specific rotations recorded for the crystalline methyl α- and β-D-arabofuranosides (+128° and -119° in water)[84] is maintained in the corresponding 3-acetamido derivatives (+134° and -120° in water). The mother liquors from this ammonolysis were not examined further.

Ammonolysis of methyl 2:3-anhydro-β-D-lyxofuranoside led to the isolation of 62% of the methyl 3-amino-3-deoxy-N-isopropylidene-β-D-arabofuranoside described by Baker.[19] The specific rotation of this compound was in exact agreement with that recorded by Baker but, although recrystallised several times, this derivative also failed to analyse well and to melt sharply. N-Acetylation, however, gave a good yield of the crystalline β N-acetyl derivative isolated in the previous experiment from the ammonolysis of the mixed methyl 2:3-anhydro-D-lyxofuranosides. Unfortunately Baker records no figure for his yield of N-isopropylidene derivative from an 'analytically'

pure sample of the β anhydro sugar but only records a 47% yield from crude material.

Once more an attempt was made to isolate a 3:5-isopropylidene xylose derivative, ~~this time~~ from the mother liquors after removal of the β N-isopropylidene-3-amino-arabinose derivative. No crystalline derivative could be obtained after acetonation and the chloroform fraction yielded a very dark, non-reducing, syrup in an overall yield of 0·6% from the crude ammonolysis product. The syrup (23% yield) isolated from the aqueous fraction gave a negative test for the isopropylidene group and yielded after distillation crystals in 0·6% yield. The crystals which gave on chromatographic analysis a single spot had constants which differed from those of methyl 3-acetamido-3-deoxy-β-D-arabofuranoside and it is presumed that they are methyl 2-acetamido-2-deoxy-β-D-xylofuranoside. Chromatographic analysis of the mother liquors gave two spots one of which was identical with that given by the crystals, although no further yield of crystals could be isolated.

Two methylations of the 2:3-anhydro-lyxofuranosides with the Purdie reagents were sufficient to give the 5-methyl ethers. The known crystalline α-anomer was obtained in good yield. The β-anomer was isolated as a very mobile syrup which, after distillation, crystallised completely on cooling in ice. The melting point was difficult to determine as it was below room temperature.

A mixture of the two anomers was obtained on methylation of 'syrupy' methyl 2:3-anhydro-αβ-D-lyxofuranoside.

From the ammonolysis product of methyl 2:3-anhydro-5-O-methyl-α-D-lyxofuranoside a crystalline N-isopropylidene derivative was isolated in 72% yield and this, like the analogous compounds from the unmethylated anhydro sugars, gave a poor melting point and analysis. N-Acetylation gave a crystalline acetate which was identified as methyl 3-acetamido-3-deoxy-5-O-methyl-α-D-arabofuranoside inasmuch as complete methylation gave crystalline methyl 3-acetamido-3-deoxy-2:5-di-O-methyl-α-D-arabofuranoside, identical with the dimethyl ether obtained from the methylation of authentic methyl 3-acetamido-3-deoxy-α-D-arabofuranoside.

Ammonolysis of the 'syrupy' methyl 2:3-anhydro-5-O-methyl-αβ-D-lyxofuranoside followed by N-acetylation led to the isolation of a crystalline derivative (21% of the N-acetylated product) which was identical with the methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside subsequently obtained and identified.

No crystalline N-isopropylidene derivative could be isolated after ammonolysis of the β 5-methyl-anhydro-sugar. However, after purification by distillation of the syrupy N-isopropylidene mixture a crystalline N-acetyl derivative was obtained (49% of the N-acetylated product). This was identified as methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside by methylation to a crystalline

dimethyl ether identical with that obtained by repeated methylation of methyl 3-acetamido-3-deoxy-β-D-arabofuranoside. During this latter methylation a crystalline mono-methyl ether was isolated, and identified, as methyl 3-acetamido-3-deoxy-2-O-methyl-β-D-arabofuranoside, by conversion to a mono-tosyl ester which gave a positive test for the presence of the primary toluene p-sulphonyloxy group. It should be mentioned that the β-derivatives of 3-acetamido-3-deoxy-D-arabinose were insoluble in methyl iodide and, although the addition of methanol brought about solution of the material, complete methylation was difficult to effect.

No crystalline xylose derivative could be isolated from the mother liquors from the ammonolysis of either the α- or the β-5-methyl-anhydro sugars, although syrups (2% yield from the crude ammonolysis product) with positive rotations were obtained after distillation of the mother liquors from the β 5-methyl anhydro sugar.

Ammonolysis undoubtedly causes decomposition for the crude product is always dark brown and this, besides depleting the yield, makes the isolation of crystalline products difficult. The percentage yields of crystalline 3-amino-arabinose derivatives, isolated in the present work, are set out below.

	3-Amino-arabinose derivatives	
	α-anomer %	β-anomer %
From 2:3-Anhydro-sugar	85	62
" 5-Methyl-2:3-anhydro-sugar	72	49

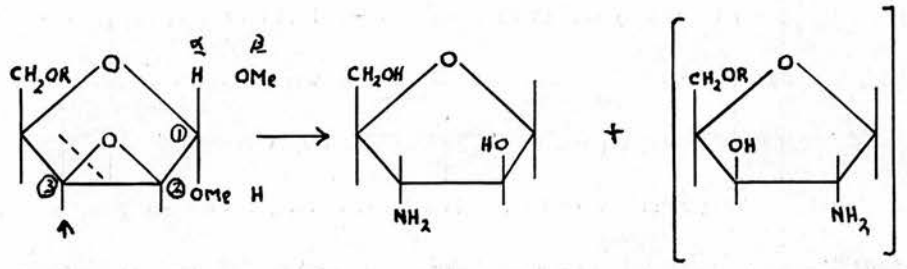
In view of the difficulties of isolation it can safely be said that these yields do not represent the total arabinose content and it is significant in this respect that where there is a lower yield there is also an increase in solubility of the isolated crystals. While no significant yields of crystalline derivatives of 2-amino-xylose could be obtained in this work there is some evidence, in the ammonolysis mother liquors, for the presence of a sugar derivative other than the arabinose derivative, but it is considered that this does not represent more than a small fraction of the products. The results of all four ammonolyses are in fact such that it may be concluded that the action of ammonia, whether it be as NH_2^- as suggested by Peat[17] or as NH_3 as suggested by Ingold,[78] favours a break between C_3 and the oxygen atom of the oxide ring and incorporation of the amino group at C_3. Certainly the yield of the 3-amino-arabinose derivative obtained from the methyl 2:3-anhydro-α-D-lyxofuranoside indicates an almost complete conversion to this sugar.

Percival and Zobrist[67] from the action of sodium methoxide on methyl 2:3-anhydro-αβ-D-lyxofuranoside isolated 3-methyl-arabinose and 2-methyl-xylose derivatives in the ratio of 2:1. Similar treatment of the 5-methyl-anhydro-sugar gave no evidence of the presence of 2:5-dimethyl-xylose although the yield of the 3:5-dimethyl-

arabinose was not very high.

It is difficult to find an explanation which will account for all the different yields of products from the fission of these 2:3-epoxide furanose sugar compounds. Furanose sugar rings are considered to be nearly planar and therefore theories advanced concerning the direction of ring fission in pyranose sugars based on the conformation taken up by the sugar ring do not apply. The importance of electronic factors in governing the direction of epoxide ring opening has long been realised and more recently the influence of steric effects exercised by substituents has been recognised.(85) It appears very probable that the direction of fission of epoxide rings in furanose sugars is influenced by both these factors. If it is assumed that at the transition stage bond breaking is more important than bond making then, in the present instance, although the epoxide ring is attached to electron attracting substituents on both sides, it is considered that the effect of the hemiacetal group is the stronger. Applying the arguments of Vanderwerf(86) this would result in preferential cleavage between C_3 and the epoxide oxygen and explain the higher proportion of arabinose derivatives formed. That this fission is apparently enhanced in the α-series can be explained as being due to

the steric effect of the α-glycosidic methoxyl group hindering attack. The larger yields of arabinose

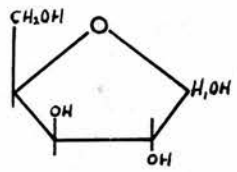


R = H or Me 3-amino-arabinose 2-amino-xylose

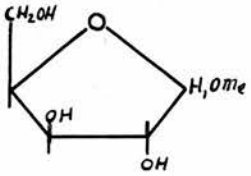
derivatives from attack by ammonia as compared with attack by sodium methoxide follow from these assumptions, for ammonia is a weaker base than OMe^- and therefore with ammonia, bond making is even less important than with OMe^-.

No reason for the smaller yield of crystalline arabinose derivatives from the 5-methyl derivatives after attack by ammonia can be advanced beyond the fact of the greater solubility of the crystals in the crystallising solvent.

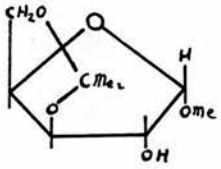
The Syntheses of Derivatives of 2:3-Anhydro-D-lyxose



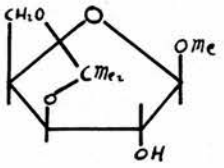
D-Xylose



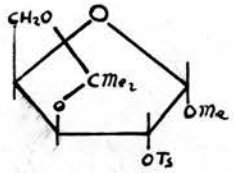
Methyl D-Xylofuranoside



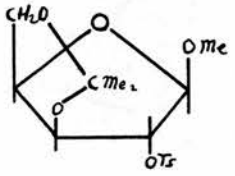
Methyl 3:5-Isopropylidene-α-D-xyloside



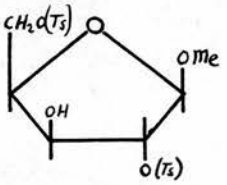
Methyl 3:5-Isopropylidene-β-D-xyloside



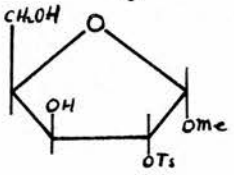
Methyl 3:5-Isopropylidene-2-tosyl-α-D-xyloside



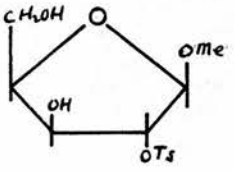
Methyl 3:5-Isopropylidene-2-tosyl-β-D-xyloside



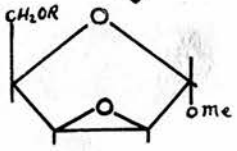
Methyl 2,5(?)-Di-tosyl-β-D-xyloside



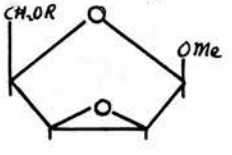
Methyl 2-Tosyl-α-D-xylofuranoside



Methyl 2-Tosyl-β-D-xylofuranoside



Methyl 2:3-Anhydro-α-D-lyxofuranoside (R=H)



Methyl 2:3-Anhydro-β-D-lyxofuranoside (R=H)

Methyl 2:3-Anhydro-5-methyl-α-D-lyxofuranoside (R=Me)

Methyl 2:3-Anhydro-5-methyl-β-D-lyxofuranoside (R=Me)

NOTES

Melting points were determined on the Kofler hot-stage microscope. Specific rotations were measured in 1 dm. polarimeter tubes. Elemental analyses for C, H, N, and S were performed by Drs. Weiler and Strauss, Oxford. Methoxyl,[79] acetyl,[80] and phosphorus[81] determinations were carried out by the author. Estimations of acetyl content, on compounds containing an N-acetyl group, were too variable to have any significance. For analysis and determination of physical constants, all compounds were dried *in vacuo* (0·1 mm.) over phosphoric oxide. All evaporations were carried out under reduced pressure at temperatures not higher than 50°. Salts of silver and barium were removed by filtration through layers of charcoal and Filtercel on a sintered glass funnel and the residue on the filter washed at least thrice with a suitable solvent. In the case of the silver salts from Purdie methylations, boiling acetone was used. All light petroleum used was the fraction of b.p. 60-80°.

The chromatographic examination of the various N-acetylated glycosides of amino pentoses was carried out using butanol-ethanol-water (4:1:5) as eluant and the sugars were detected according to the method Rydon and Smith[87] with chlorine and starch-potassium iodide. The R_G values were calculated with tetra-methyl-glucose (R_G 1·0) as standard.

The analyses of compounds, new when prepared by the author, are reported in the form

Found: C, -,-; H, -.-.

$C_6H_{12}O_6$ requires C, -.-; H, -.-%.

whereas for compounds which are mixtures of anomers or which had already been adequately described in the literature the form is

Found: C, -.-; H, -.-.

Calc. for $C_6H_{12}O_6$: C, -.-; H, -.-%.

Throughout the experimental sections, the nomenclature is in accordance with the recommendations of the Joint Committee on Nomenclature.[82] In the Introduction and Discussion however, the nomenclature is simplified for the sake of clarity; the generally recognised trivial name D-glucosamine is used throughout instead of the systematic name 2-amino-2-deoxy-D-glucose.

The Syntheses of the Methyl 2:3-Anhydro-D-lyxofuranosides and their 5-O-Methyl Ethers.

Methyl D-Xylofuranoside.

Dry crystalline D-xylose (22 g.) was treated with dry methanolic hydrogen chloride (500 ml., 0·5%) for 5 hours at room temperature.(64) Neutralisation with silver carbonate followed by filtration and evaporation gave a colourless syrup, which was exhaustively extracted with ethyl acetate. The combined extracts were concentrated to a colourless syrup (18·2 g., 76% of theoretical) which had $[\alpha]_D^{18}$ +21° (c, 1·0 in EtOH).

Methyl 3:5-O-Isopropylidene-D-xylofuranoside.

Methyl xylofuranoside (18·0 g.) was shaken with dry acetone (600 ml.), acetaldehyde (0·20 ml.) and anhydrous copper sulphate (60 g.) for 4 days. Concentrated sulphuric acid (0·4 ml.) was added and shaking continued for a further 24 hours.(65) The copper sulphate was removed by filtration and washed thoroughly with dry acetone. The combined acetone solutions were neutralised with barium carbonate, filtered, and evaporated to a mobile syrup. Distillation at 110-130° (bath temperature) /0·05 mm. gave a colourless mobile syrup (16·8 g., 75% of theoretical) which had $[\alpha]_D^{15}$ +20° (c, 1·0 in H_2O), n_D^{15} 1·4640. (Percival and Zobrist(66) record $[\alpha]^{15}$ -26° in H_2O n_D^{15} 1·4640).

Found: acetone, 29·1.

Calc. for $C_9H_{16}O_5$: acetone, 28·4%.

Methyl 3:5-O-Isopropylidene-2-O-toluene-p-sulphonyl-D-xylofuranoside.

To methyl 3:5-O-isopropylidene-D-xyloside (16·8 g.) in pyridine (45 ml.), powdered toluene-p-sulphonyl chloride (23·0 g.) was added and the mixture kept at room temperature for 48 hours in the presence of 'Drierite' (2 g.).[66] The filtered reaction solution was poured slowly into stirred ice-water and the precipitate which formed was washed with ice-water until free from pyridine. The product was a white powder (19·2 g., 65% of theoretical), $[\alpha]_D^{19}$ +10° (c, 1·3 in $CHCl_3$).

Methyl 2-O-Toluene-p-sulphonyl-D-xylofuranoside.

Methyl 3:5-O-isopropylidene-2-O-toluene-p-sulphonyl-D-xyloside (19·2 g.) was treated with dry 1% methanolic hydrogen chloride (100 ml.) at 70° for 1 hour.[66] Neutralisation with silver carbonate, filtration, and evaporation gave a pale syrup (15·4 g., 91% of theoretical), $[\alpha]_D^{15}$ +37° (c, 0·8 in MeOH) n_D^{17} 1·5210. (Percival and Zobrist[66] record $[\alpha]_D^{15}$ +41° in MeOH, n_D^{15} 1·5269).

Methyl 2:3-Anhydro-D-lyxofuranoside.

Methyl 2-O-toluene-p-sulphonyl-D-xylofuranoside (15·4 g.) in ethanol (300 ml.) at 75° was titrated with 2N sodium hydroxide solution until the mixture was permanently pink to phenolphthalein (22·1 ml.).[67] The solution was evaporated to dryness and the residue exhaustively extracted with cold

ethyl acetate. After removal of the insoluble sodium toluene-p-sulphonate the filtrate was concentrated to a syrup (6·9 g., 98% of theoretical). On standing at 0°, this partly crystallised. The crystals (1·8 g., 25% of theoretical) were identical with the methyl 2:3-anhydro-α-D-lyxofuranoside prepared by Percival and Zobrist.[67] After one recrystallisation from benzene they had m.p. 79°, mixed m.p. with authentic material 79-80°, $[\alpha]_D^{17}$ +52° (c, 0·4 in H_2O). (Percival and Zobrist record m.p. 81°, $[\alpha]_D^{15}$ +57° in H_2O).

The residual syrup was freed from water insoluble impurities and the aqueous solution on concentration gave a syrup (3·5 g.), $[\alpha]_D^{18}$ -49° (c, 1·1 in H_2O). (Percival and Zobrist[67] record $[\alpha]_D^{15}$ +4° in H_2O). This product is considered to be an α-β mixture of the desired anhydro compound.

Fractional Distillation of Methyl 3:5-O-Isopropylidene-D-xylofuranoside.

Methyl 3:5-O-isopropylidene-D-xylofuranoside was prepared (from D-xylose (200 g.)) as described on p. 33 The product was purified before distillation by solution in water (200 ml.) and extraction with chloroform (3 x 200 ml.). The dried chloroform extracts were concentrated to a colourless syrup (172 g.). This syrup was fractionally distilled (in 30 g. portions) with a Vigreux column and the specific rotation of each fraction was used as an indication of the extent of fractionation. If this value lay between the arbitrarily set standards, $[\alpha]_D$ $+65^\circ \rightarrow -65^\circ$, then the product was re-fractionated. Finally, two fractions were obtained.

Fraction I: 71·6 g. (42% of theoretical), b.p. range 60-70°/0·05 mm., average $[\alpha]_D$ +75° (c, 1·0 in $CHCl_3$), (range of $[\alpha]_D$ $+70^\circ \rightarrow +82^\circ$), n_D^{18} 1·4638.

Fraction II: 55·2 g. (32% of theoretical), b.p. range 90-100°/0·05 mm., average $[\alpha]_D$ -80° (c, 1·0 in $CHCl_3$), (range of $[\alpha]_D$ $-65^\circ \rightarrow -85^\circ$), n_D^{17} 1·4650.

Fraction I was believed to be predominantly methyl 3:5-O-isopropylidene-α-D-xylofuranoside and fraction II predominantly methyl 3:5-O-isopropylidene-β-D-xylofuranoside.

Baker, Schaub, and Williams[19] carried out this fractionation and obtained fractions (I) b.p. 85-88°/0·1 mm., $[\alpha]_D^{24}$ +18° in water and (II) b.p. 108-110°/0·1 mm., $[\alpha]_D^{24}$ -64° in water.

Methyl 3:5-O-Isopropylidene-2-O-toluene-p-sulphonyl-α-D-xylofuranoside.

A cooled solution of methyl 3:5-O-isopropylidene-D-xylofuranoside (71 g., $[\alpha]_D$ +75°) in dry pyridine (120 ml.) mixed with toluene-p-sulphonyl chloride (73 g.) in the presence of 'Drierite' (7 g.) was allowed to stand at room temperature for 24 hours. The mixture was then filtered and ice-water (10 ml.) added to decompose the excess of acid chloride. The resulting solution was poured into stirred ice-water (3 l.). After stirring for 3 hours, the precipitated crystalline solid was separated and dried. This product (92 g., 74% of theoretical) which had m.p. 77°, $[\alpha]_D^{21}$ +66° (c, 1·4 in $CHCl_3$) was considered to be sufficiently pure for further use. This material, methyl 3:5-O-isopropylidene-2-O-toluene-p-sulphonyl-α-D-xylofuranoside, after recrystallisation from light petroleum, had m.p. 79-80°, $[\alpha]_D^{18}$ +68° (c, 1·1 in $CHCl_3$).

Found: C, 53·2; H, 6·1; S, 8·9.

$C_{16}H_{22}O_7S$ requires C, 53·6; H, 6·2; S, 9·0%.

Methyl 3:5-O-Isopropylidene-2-O-toluene-p-sulphonyl-β-D-xylofuranoside.

Methyl 3:5-O-isopropylidene-D-xylofuranoside (55 g., $[\alpha]_D$ -80°) in dry pyridine (100 ml.) was treated as in the previous experiment with toluene-p-sulphonyl chloride (57 g.). The product isolated (80 g.) had $[\alpha]_D^{18}$ -37° (c, 1·1 in $CHCl_3$). Recrystallisation from aqueous methanol gave methyl 3:5-O-

isopropylidene-2-O-toluene-p-sulphonyl-β-D-xylofuranoside (62 g., 64% of theoretical), m.p. 119-120°, $[\alpha]_D^{17}$ -53° (c, 0.9 in $CHCl_3$), $[\alpha]_D^{18}$ -44° (c, 0.4 in MeOH). (Percival and Zobrist[66] record m.p. 120°, $[\alpha]_D^{15}$ -45° in MeOH for this product).

Methyl 2-O-Toluene-p-sulphonyl-α-D-xylofuranoside.

Preliminary Experiment. Methyl 3:5-O-isopropylidene-2-O-toluene-p-sulphonyl-α-D-xylofuranoside (203 mg.) was allowed to stand with 0·1% methanolic hydrogen chloride (20 ml.) at room temperature until the rotation of the solution reached a constant value.

Time (min.)	α_D (2 dm.)
0	+ 1·46°
3	+ 1·80°
5	+ 1·95°
10	+ 2·08°
15	+ 2·12°
20	+ 2·13°
25	+ 2·13°

Neutralisation with silver carbonate followed by filtration and evaporation gave a syrup which crystallised completely. Recrystallisation from chloroform-light petroleum gave methyl 2-O-toluene-p-sulphonyl-α-D-xylofuranoside (144 mg., 80% of theoretical), m.p. 90-91°,

$[\alpha]_D^{25}$ +101° (c, 1·3 in $CHCl_3$).

Found: C, 49·5; H, 5·8; S, 10·4.

$C_{13}H_{18}O_7S$ requires C, 49·1; H, 5·7; S, 10·1%.

Large Scale Preparation. Methyl 3:5-O-isopropylidene-2-O-toluene-p-sulphonyl-α-D-xylofuranoside (89 g.) was treated as in the previous experiment with 0·1% methanolic hydrogen chloride (2200 ml.). The rotation was constant after 40 minutes and the product, isolated as a syrup (75 g., 95% of theoretical), crystallised completely. This was considered to be sufficiently pure for further use without recrystallisation. It had m.p. 88-90°, $[\alpha]_D^{24}$ +97° (c, 1·2 in $CHCl_3$).

Methyl 2-O-Toluene-p-sulphonyl-β-D-xylofuranoside.

Preliminary Experiments. (a) Methyl 3:5-O-isopropylidene-2-O-toluene-p-sulphonyl-β-D-xylofuranoside was treated with 1% methanolic hydrogen chloride at room temperature and an attempt was made to follow the hydrolysis of the isopropylidene group by estimating the acetone liberated.(68) However, the methanol present in the aliquots also reacted with the alkaline hypoiodite and the method was abandoned.

(b) The β-isopropylidene derivative (519 mg.) in 80% acetic acid (37·5 ml.) was heated at 50°.(19) After 30 minutes the rotation of the solution was found to have reached a constant value. On cooling, needle shaped crystals (40 mg.) separated and were removed. No further yield of

crystals could be obtained from the mother liquors. After recrystallisation from methanol, the crystals had m.p. 132-133°, $[\alpha]_D^{20}$ -32° (c, 1·3 in $CHCl_3$).

Found: C, 51·3; H, 4·9; S, 14·9.

Calc. for $C_{13}H_{18}O_7S$: C, 49·1; H, 5·7; S, 10·1.

Calc. for $C_{20}H_{24}O_7S_2$: C, 50·8; H, 5·1; S, 13·6%.

($C_{13}H_{18}O_7S$ = Methyl 2-O-toluene-p-sulphonyl-β-D-xylofuranoside $C_{20}H_{24}O_7S_2$ = Methyl di-O-toluene-p-sulphonyl-β-D-xylofuranoside). The crystals gave a positive test for the presence of a primary toluene-p-sulphonyloxy group[63] and this, together with the analytical figures, indicated that the material was a di-tosyl ester, possibly methyl 2,5-di-O-toluene-p-sulphonyl-β-D-xylofuranoside.

(c) The β-isopropylidene derivative (251 mg.) with 1% methanolic hydrogen chloride (25 ml.) was kept at room temperature until the rotation of the solution reached a constant value (5 minutes). Neutralisation with silver carbonate, filtration, and evaporation gave a syrup which could not be crystallised.

(d) The β-isopropylidene derivative (3·6 g.) after standing with 0·1% methanolic hydrogen chloride (400 ml.) at room temperature until the rotation of the solution reached a constant value, gave, after the usual treatment, a

Time (min.)	α_D (2 dm.)
0	-0.83°
3	-0.78°
10	-0.60°
15	-0.53°
20	-0.51°
25	-0.50°

syrup. This on solution in hot methanol and cooling, gave a small quantity (280 mg.) of the di-tosyl ester previously obtained. The residual syrup, methyl 2-O-toluene-p-sulphonyl-β-D-xylofuranoside, had $[\alpha]_D^{22}$ -20° ($CHCl_3$), n_D^{22} 1·5243.

Large Scale Preparation. The β-isopropylidene derivative (54 g.) with 0·1% methanolic hydrogen chloride (4300 ml.) gave in a preparation similar to the preliminary experiment (d), syrupy methyl 2-O-toluene-p-sulphonyl-β-D-xylofuranoside (45·5 g., 95% of theoretical).

Methyl 2:3-Anhydro-α-D-lyxofuranoside.

Methyl 2-O-toluene-p-sulphonyl-α-D-xylofuranoside (75 g.) in ethanol (800 ml.) at 75° was titrated with 2N sodium hydroxide solution (103 ml.) as previously described (p.34). Evaporation of the ethyl acetate extracts gave a crystalline residue (34·3 g.) which on recrystallisation from carbon tetrachloride gave methyl 2:3-anhydro-α-D-lyxofuranoside (29·3 g., 85% of the theoretical), m.p. 80°, $[\alpha]_D^{21}$ +60° (c, 1·0 in H_2O).

Methyl 2:3-Anhydro-β-D-lyxofuranoside.

Methyl 2-O-toluene-p-sulphonyl-β-D-xylofuranoside (45 g.) on similar treatment with 2N sodium hydroxide solution (68 ml.) gave a crystalline residue (19·8 g.) which after recrystallisation from benzene-light petroleum gave methyl 2:3-anhydro-β-D-lyxofuranoside (17·6 g., 85% of theoretical), with m.p. 73-74°, and $[\alpha]_D^{18}$ -104° (c, 1·0 in H_2O). (Baker, Schaub, and Williams[19] record m.p. 74-75°, $[\alpha]_D^{25}$ -102°).

Found: C, 49·6; H, 6·7.

Calc. for $C_6H_{10}O_4$: C, 49·3; H, 6·9%.

Methyl 2:3-Anhydro-5-O-methyl-α-D-lyxofuranoside.

Methyl 2:3-anhydro-α-D-lyxofuranoside (11·7 g.) in dry methanol (5 ml.) was methylated at 40° with methyl iodide (50 ml.) and silver oxide (48 g.), the latter being added in portions over 6 hours. The silver residues were removed and thoroughly washed with hot dry acetone. The combined filtrate and washings were concentrated at room temperature. Remethylation of the crystalline residue as before (without addition of methanol) gave a crystalline product (10·6 g.) which on purification by sublimation at 40°/0·01 mm. gave crystals of methyl 2:3-anhydro-5-O-methyl-α-D-lyxofuranoside (9·8 g., 76% of theoretical), m.p. 41-42°, $[\alpha]_D^{26}$ +61° (c, 1·4 in H_2O), $[\alpha]_D^{26}$ +67° (c, 1·2 in MeOH). (Percival and Zobrist[67] record m.p. 43°, $[\alpha]_D^{18}$ +60° in MeOH).

Methyl 2:3-Anhydro-5-O-methyl-β-D-lyxofuranoside.

Methyl 2:3-anhydro-β-D-lyxofuranoside (8.0 g.) in dry methanol (10 ml.) was methylated three times with methyl iodide (50 ml.) and silver oxide (48 g.) as in the previous experiment. The reaction was followed by noting the change in the refractive index.

After 1 methylation		n_D^{21} = 1·4590
" 2	"	n_D^{21} = 1·4478
" 3	"	n_D^{21} = 1·4478

The final product was a very mobile syrup (7·3 g.), $[\alpha]_D^{20}$ -84° (c, 0·7 in H_2O). Distillation gave a syrup (6·4 g., 73% of theoretical), b.p. 54-57°/0·05 mm., $[\alpha]_D^{20}$ -88° (c, 1·1 in H_2O), n_D^{21} 1·4470.

Found: C, 52·8; H, 7·8; OMe, 39·1.

$C_7H_{12}O_4$ requires C, 52·5; H, 7·6; OMe, 38·8%.

This syrup, methyl 2:3-anhydro-5-O-methyl-β-D-lyxofuranoside, crystallised completely on cooling to 0° but melted below room temperature (m.p. ca. 14-15°).

'Syrupy' Methyl 2:3-Anhydro-5-O-methyl-D-lyxofuranoside.

'Syrupy' methyl 2:3-anhydro-D-lyxofuranoside (4·0 g., $[\alpha]_D^{18}$ -49°) was methylated three times with methyl iodide (30 ml.) and silver oxide (20 g.) as before. The reaction was followed by noting the change in the refractive index.

Starting material		n_D^{13} 1·4730
After 1 methylation		n_D^{13} 1·4500
" 2	"	n_D^{13} 1·4503
" 3	"	n_D^{13} 1·4503

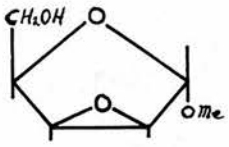
The final product was a very mobile syrup (3·9 g., 89% of theoretical), $[\alpha]_D^{15}$ -38° (c, 0·9 in H_2O). (Percival and Zobrist[67] record $[\alpha]_D^{18}$ +24° in H_2O).

Found: OMe, 38·4.

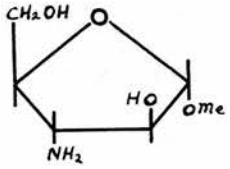
Calc. for $C_7H_{12}O_4$: OMe, 38·8%.

This syrup is presumed to be a mixture of the two anomers of methyl 2:3-anhydro-5-O-methyl-D-lyxofuranoside.

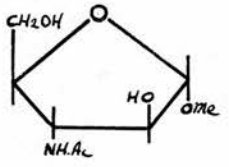
Derivatives of Methyl 3-Amino-3-deoxy-α-D-arabofuranoside



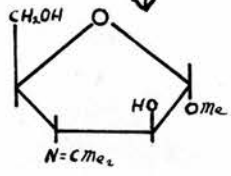
Methyl 2:3-Anhydro-α-D-lyxofuranoside



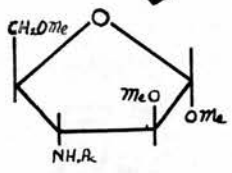
Methyl 3-Amino-3-deoxy-α-D-arabofuranoside



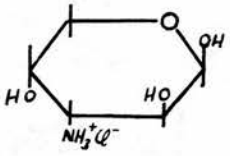
Methyl 3-Acetamido-3-deoxy-α-D-arabofuranoside



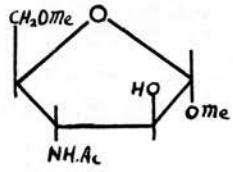
Methyl 3-Amino-3-deoxy-N-isopropylidene-α-D-arabofuranoside



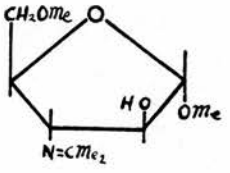
Methyl 3-Acetamido-3-deoxy-2,5-di-methyl-α-D-arabofuranoside



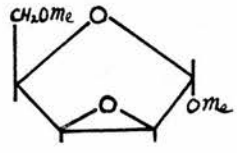
3-Amino-3-deoxy-β-D-arabinose hydrochloride



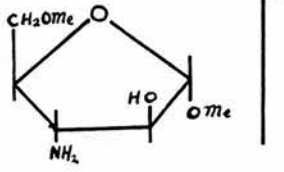
Methyl 3-Acetamido-3-deoxy-5-methyl-α-D-arabofuranoside



Methyl 3-Amino-3-deoxy-N-isopropylidene-5-methyl-α-D-arabofuranoside

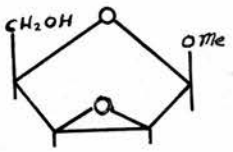


Methyl 2:3-Anhydro-5-methyl-α-D-lyxofuranoside

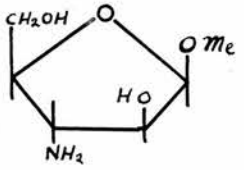


Methyl 3-Amino-3-deoxy-5-methyl-α-D-arabofuranoside

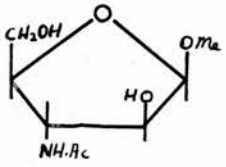
Derivatives of Methyl 3-Amino-3-deoxy-β-D-arabofuranoside



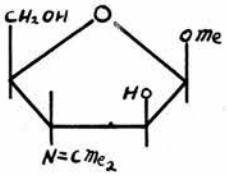
Methyl 2:3-Anhydro-β-D-lyxofuranoside



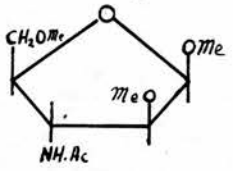
Methyl 3-Amino-3-deoxy-β-D-arabofuranoside



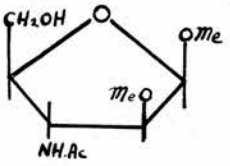
Methyl 3-Acetamido-3-deoxy-β-D-arabofuranoside



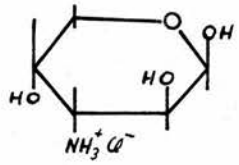
Methyl 3-Amino-3-deoxy-N-isopropylidene-β-D-arabofuranoside



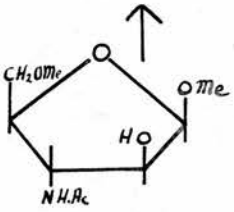
Methyl 3-Acetamido-3-deoxy-2,5-di-methyl-β-D-arabofuranoside



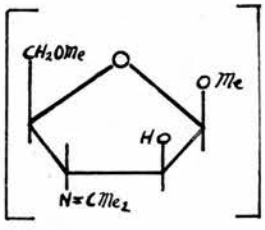
Methyl 3-Acetamido-3-deoxy-2-methyl-β-D-arabofuranoside



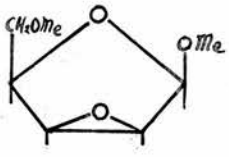
3-Amino-3-deoxy-β-D-arabinose hydrochloride



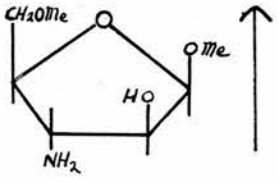
Methyl 3-Acetamido-3-deoxy-5-methyl-β-D-arabofuranoside



Methyl 3-Amino-3-deoxy-N-isopropylidene-5-methyl-β-D-arabofuranoside



Methyl 2:3-Anhydro-5-methyl-β-D-lyxofuranoside



Methyl 3-Amino-3-deoxy-5-methyl-β-D-arabofuranoside

The Ammonolysis of Methyl 2:3-Anhydro-α-D-lyxofuranoside.

(A) Preliminary Experiment

(i) Ammonolysis Crystalline methyl 2:3-anhydro-α-D-lyxofuranoside (1·40 g.) in a saturated solution of ammonia in dry methanol (65 ml.) was heated in a sealed tube at 120° for 48 hours. The resulting solution was filtered and concentrated to a brown syrup (1·55 g., 99% of theoretical). All attempts to obtain a crystalline product from this methyl α-D-pentosaminide mixture (I) failed. Part of this product (1·0 g.) after distillation gave a colourless syrup (0·85 g.), b.p. 150-170° (bath temperature)/ 0·01 mm.

(ii) N-Acetylation. The distilled methyl α-D-pentosaminide mixture (0·85 g.) in water (20 ml.) and methanol (2 ml.) at 5° was stirred with anion exchange resin (Amberlite I.R.400; bicarbonate form) (24 ml.) and acetic anhydride (0·6 ml.) for 90 minutes.[36] Filtration and evaporation gave a syrup (0.96 g., 90% of theoretical) which on solution in methanol-acetone (1:1) and addition of ether to turbidity gave a crystalline product (0.524 g., 54% of the crude N-acetylated product) which had m.p. 110-115° and was shown to be methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (II), These crystals on recrystallisation from acetone had m.p. 120-121°, $[\alpha]_D^{22}$ +134° (c, 0·9 in H_2O), $[\alpha]_D^{22}$ +124° (c, 0·7 in EtOH). (Baker, Schaub,

and Williams[19] record m.p. 115-116°, $[\alpha]_D^{24}$ +102° in H_2O).
Found: C, 47·2; H, 7·6; N, 6·5.
$C_8H_{15}O_5N$ requires C, 46·8; H, 7·4; N, 6·8%.

(iii) Attempted formation of an isopropylidene derivative. Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (II) (50 mg.) was shaken with dry acetone (10 ml.), anhydrous copper sulphate (1 g.) and acetaldehyde (0·1 ml.) for 14 days. Filtration and evaporation of the filtrate gave a syrup (45 mg.) which on trituration with acetone crystallised completely. This product was unchanged starting material, m.p. and mixed m.p. with starting material 118-120°, $[\alpha]_D^{18}$ +136° (c, 1·0 in H_2O).
Found: C, 46·9; H, 7·4.
$C_8H_{15}O_5N$ requires C, 46·8; H, 7·4.
Calc. for $C_{11}H_{19}O_5N$ (isopropylidene derivative) C, 53·9; H, 7·8%.

(iv) Tosylation. Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (II) (10 mg.) in dry pyridine (1 ml.) was treated with toluene-p-sulphonyl chloride (40 mg.) in the presence of 'Drierite' (0·1 g.) for 60 hours at room temperature. The reaction mixture was filtered, the excess of acid chloride decomposed with a few drops of ice-water, and the mixture poured slowly into stirred ice-water. The resulting precipitate after washing with water and drying gave methyl 3-acetamido-3-deoxy-2,5-di-O-toluene-p-sulphonyl-α-D-arabofuranoside (III) as a white powder (8 mg.).

(v) Treatment of the ditosyl derivative with sodium iodide. The ditosyl derivative (III) (6 mg.), in dry acetone (0·1 ml.) was heated with sodium iodide (10 mg.) in a sealed tube at 100° for 2 hours.[63] Characteristic plate-shaped crystals of sodium toluene-p-sulphonate were deposited in 88% yield (after allowance[89] for solubility in acetone) and were identified as the S-benzylthiouronium salt, m.p. 179-180°. (Hopkins and Williams[71] record m.p. 182°). The yield of sodium toluene-p-sulphonate was comparable to that obtained from a corresponding quantity of methyl 2,3,4-tri-O-benzoyl-6-O-toluene-p-sulphonyl-α-D-mannopyranoside (81% of theoretical). Toluene-p-sulphonamide under these same conditions failed to yield any sodium toluene-p-sulphonate.

(vi) Hydrolysis of the methyl α-D-pentosaminide mixture. Crude methyl α-D-pentosaminide (I) (135 mg.) in N sulphuric acid (11 ml.) was heated at 100° until the rotation of the solution reached a constant value.

Time (min.)	α_D (1 dm.)
15	+0·45°
30	+0·28°
60	+0·00°
105	-0·30°
145	-0·48°
180	-0·53°
240	-0·53°

Neutralisation with barium carbonate, filtration, and concentration gave a syrup which was extracted with water (3x1 ml.). The aqueous extracts were filtered and evaporated to a syrup (80 mg., 65% of theoretical), $[\alpha]_D^{19}$ -47° (c, 1·1 in H_2O). This pentosamine mixture could not be crystallised and no crystalline hydrochloride could be isolated.

(vii) Hydrolysis of methyl 3-acetamido-3-deoxy-α-D-arabofuranoside. Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (II) (91 mg.) in 3N hydrochloric acid (10 ml.) was heated at 100° until the rotation of the solution reached a constant value.

Time (min.)	α_D (1 dm.)
0	+1.24°
15	-0·87°
35	-0·86°
60	-0·86°

The hydrolysis solution was evaporated to dryness and the residue dried by treatment with ethanol-benzene (3:1) and removal of the solvents. Trituration with methanol gave crystals of 3-amino-3-deoxy-β-D-arabinose hydrochloride (69 mg., 84% of theoretical), m.p. 161° (dec.), $[\alpha]_D^{18}$ -143° (3 minutes) ⟶ $[\alpha]_D^{18}$ -109° (final; 30 minutes) (c, 0·3 in H_2O). (Baker, Schaub, and Williams[19] record m.p. 159° (dec.) $[\alpha]_D^{24}$ -110°, and -112° in H_2O).

Found: C, 32·6; H, 6·5; N, 7·3; Cl, 18·7.
$C_5H_{11}O_4N.HCl$ requires C, 32·4; H, 6·5; N, 7·6; Cl, 19·1%.

(B) Large Scale Preparation.

(i) Ammonolysis. Methyl 2:3-anhydro-α-D-lyxofuranoside (13·2 g.) in methanolic ammonia (500 ml.) was heated in an autoclave at 120° for 48 hours. The resulting brown solution was filtered and treated twice with charcoal at room temperature. Removal of the solvent gave a brown syrup which was freed from ammonia by solution in ethanol and concentration to a syrup (I) (14·60 g., 99% of theoretical).

(ii) Separation of a crystalline N-isopropylidene derivative. The crude ammonolysis product (I) was dissolved in hot acetone (250 ml.) and the cooled solution deposited crystals of methyl 3-amino-3-deoxy-N-isopropylidene-α-D-arabofuranoside (IV). The mother liquors were concentrated and treated several times in this way and a total yield of 15·5 g. (85% of theoretical) of crystalline material was obtained.

After several recrystallisations from acetone, this compound had m.p. 120-135°, $[\alpha]_D^{25}$ +100° (c, 1·7 in H_2O). (Baker[19] records m.p. 157-159°, $[\alpha]_D^{26}$ +98·5° in H_2O).
Found: C, 49·8; H, 8·2; N, 7·1.
Calc. for $C_9H_{17}O_4N$: C, 53·2; H, 8·4; N, 6·9.

Calc. for $C_6H_{13}O_4N$: C, 44·2; H, 8·0; N, 8·6%.

($C_6H_{13}O_4N$ = Methyl 3-amino-3-deoxy-α-D-arabofuranoside).

The mother liquors were concentrated to a syrup (V) (1·8 g.).

(iii) N-Acetylation of the crystalline N-isopropylidene derivative. A solution of the crystalline N-isopropylidene derivative (IV) (8·0 g.) in water (200 ml.) was acidified with 2N hydrochloric acid to hydrolyse the isopropylidene group and then cooled to 5° with methanol (20 ml.) and anion exchange resin (Amberlite I.R.400; bicarbonate form) (200 ml.). Acetic anhydride (5·5 ml.) was added and the mixture stirred for 90 minutes at 5°. Removal of the resin, followed by evaporation, gave a syrup which on solution in ethanol and addition of light petroleum deposited crystals (6·62 g., 82% of theoretical), m.p. 120-121°, identical with the methyl 3-acetamido-3-deoxy-α-D-arąbo-furanoside (II) previously prepared.

(iv) Investigation of the residual syrup (V). After removal of the crystalline N-isopropylidene derivative (IV) from the ammonolysis product, the residual material (V) (1·8 g.) in water (50 ml.) was N-acetylated in the usual way with methanol (5 ml.), resin (50 ml.), and acetic anhydride (1·25 ml.). The syrupy product (1·5 g., 83% of theoretical) in dry acetone (50 ml.) was shaken with anhydrous copper sulphate (8 g.) and N sulphuric acid (0·08 ml.).[19] After 3 days the copper sulphate was

removed and the solution neutralised by addition of ammonium hydroxide. Evaporation gave a non-reducing syrup (1·0 g.) which was fractionated between chloroform and water by a counter-current technique. A syrup (900 mg., 90% of theoretical) was obtained on evaporation of the aqueous fraction which had $[\alpha]_D^{22}$ +112° (c, 1·1 in H_2O), (c.f. $[\alpha]_D$ +134° in H_2O for methyl 3-acetamido-3-deoxy-α-D-arabofuranoside). It was non-reducing to Fehling's solution before, and reducing after, hydrolysis with hot dilute hydrochloric acid, and gave a negative test for the presence of the isopropylidene group.(68) Chromatographic examination (see p. 31) showed the presence of two major components, R_G 0·67 and R_G 0·76 (c.f. R_G 0·73 for methyl 3-acetamido-3-deoxy-α-D-arabofuranoside). No crystalline fraction could be obtained from this product.

Evaporation of the chloroform fraction yielded a syrup (40 mg., 4% of theoretical) which was non-reducing to Fehling's solution and which gave a faint positive test for the presence of the isopropylidene group.

Methylation of methyl 3-acetamido-3-deoxy-α-D-arabofuranoside.

Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (II) (4·0 g.) was methylated in the usual way with methyl iodide (23 ml.) and silver oxide (20 g.). The reaction was followed by noting the change in the refractive index.

After 1 methylation n_D^{20} 1·4788

After 2 methylations n_D^{20} 1·4713

After 3 methylations n_D^{20} 1·4654

After 4 methylations n_D^{20} 1·4652

The product was a mobile syrup (4·30 g.) which after distillation, b.p. 50-120°/0·05 mm., had n_D^{20} 1·4647, and on standing partly crystallised. The crystals (2·69 g., 59% of theoretical) were freed from syrup by washing with cold light petroleum. These hygroscopic crystals of methyl 3-acetamido-3-deoxy-2,5-di-O-methyl-α-D-arabofuranoside. could not be recrystallised and had m.p. 57-59°, $[\alpha]_D^{23}$ +131° (c, 1·0 in H_2O), and R_G 0·97.

Found: C, 51·6 ; H, 8·1 ; N, 5·8 ; OMe, 39.2.

$C_{10}H_{19}O_5N$ requires C, 51·5; H, 8·2; N, 6·0; OMe, 39·9%.

The Ammonolysis of 'Syrupy' Methyl 2:3-Anhydro-D-lyxofuranoside.

(i) Ammonolysis. 'Syrupy' methyl 2:3-anhydro-D-lyxofuranoside (2·8 g.) in methanolic ammonia (75 ml.) was heated in a sealed tube at 120° for 48 hours. The resulting brown solution was concentrated to a syrup which was treated with charcoal in ethanol. Removal of the solvent gave a syrup (VI) (2·81 g., 90% of theoretical).

(ii) N-Acetylation. The ammonolysis product (VI) (1·01 g.) in methanol (20 ml.) and acetic anhydride (2·5 ml.) was allowed to stand at room temperature overnight. Dilution with water (10 ml.), neutralisation with solid sodium bicarbonate, and removal of the solvent gave a residue which was extracted with chloroform (3 x 80 ml.). Concentration of the dried extracts gave a syrup (0·76 g., 59% of theoretical) which on solution in methanol-acetone (1:1) and addition of ether to turbidity gave crystals (160 mg., 17% of theoretical). These had m.p. 147-149° and were shown to be methyl 3-acetamido-3-deoxy-β-D-arabofuranoside (VII). After recrystallisation from the same solvents they had m.p. 156°, mixed m.p. with the α anomer (II) 90-100°, $[\alpha]_D^{18}$ -165° (c, 1·0 in EtOH), -120° (c, 1.2 in H_2O). (Baker[19] records m.p. 155°, $[\alpha]_D^{24}$ -119° in H_2O, for this compound.)

Found: C, 46·2; H, 7·3; N, 6·9.

$C_8H_{15}O_5N$ requires C, 46·8; H, 7·4; N, 6·8%.

The mother liquors were re-treated with methanol-acetic anhydride but no crystalline material could be obtained from the product which had $[\alpha]_D^{18}$ -4° (c, 1·5 in H_2O).

(iii) Attempted formation of an isopropylidene derivative. Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside (VII) (50 mg.) in dry acetone (10 ml.) was shaken with anhydrous copper sulphate (1 g.) and acetaldehyde (2 drops) for 14 days. Filtration and concentration gave a syrup (46 mg.) which on trituration with acetone crystallised completely. This product was unchanged starting material, m.p. and mixed m.p. with starting material 156°, $[\alpha]_D^{18}$ -160° (c, 0·5 in EtOH).

Found: C, 46·9; H, 7·4.

$C_8H_{15}O_5N$ requires C, 46·8; H, 7·4%.

Calc. for $C_{11}H_{19}O_5N$ (isopropylidene derivative): C, 53·9; H, 7·8%.

(iv) Tosylation. Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside (VII) (10 mg.) in dry pyridine (1 ml.) was treated with toluene-p-sulphonyl chloride (40 mg.) in exactly the same as for the α anomer. The ditosyl derivative (VIII) was isolated as a white powder (11 mg.).

(v) Treatment of the ditosyl derivative with sodium iodide. The ditosyl derivative (VIII) (10 mg.) in dry acetone (0·1 ml.) was heated with sodium iodide (10 mg.) in a sealed tube at 100° for 2 hours.(63) Characteristic

plate-shaped crystals of sodium toluene-p-sulphonate were deposited in 85% yield.

(vi) Hydrolysis of the ammonolysis product (VI). The crude ammonolysis product (VI) (315 mg.) in N hydrochloric acid (25 ml.) was heated at 100° until the rotation of the solution reached a constant value.

Time (min.)	α_D (1 dm.)
0	-0·25°
45	-0.60°
90	-0·68°
120	-0·68°

Evaporation gave a syrup (354 mg., 99% of theoretical), $[\alpha]_D^{21}$ -47° (c, 1·2 in H_2O), which could not be crystallised.

(vii) Hydrolysis of methyl 3-acetamido-3-deoxy-β-D-arabofuranoside. Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside (VII) (40 mg.) was hydrolysed with 3N hydrochloric acid (5 ml.) at 100°. After 1 hour, evaporation of the hydrolysate gave a syrup which on trituration with methanol gave crystals (27 mg., 75% of theoretical), m.p. 161° (dec.), $[\alpha]_D^{18}$ -108° (c, 0·7 in H_2O), identical with the 3-amino-3-deoxy-β-D-arabinose hydrochloride previously obtained.

The Ammonolysis of Methyl 2:3-Anhydro-β-D-lyxofuranoside.

(i) Ammonolysis. Crystalline methyl 2:3-anhydro-β-D-lyxofuranoside (8.4 g.) in methanolic ammonia (500 ml.) was heated in an autoclave at 120° for 48 hours. The resulting brown solution was filtered and treated twice with charcoal at room temperature. Filtration and removal of the solvent gave a brown syrup from which any residual ammonia was removed as before and a syrup (X) (9·21 g., 98% of theoretical) was isolated.

(ii) Separation of a crystalline N-isopropylidene derivative. The crude ammonolysis product (X) (9·2 g.) was dissolved in hot acetone (50 ml.) and the cooled solution deposited crystals of methyl 3-amino-3-deoxy-N-isopropylidene-β-D-arabofuranoside (XI) (total yield, 7·1 g., 62% of theoretical). After several recrystallisations from acetone, this compound had m.p. 130-145°, $[\alpha]_D^{25}$ -96° (c, 1·5 in H_2O). (Baker[19] records m.p. 155-157°, $[\alpha]_D^{25}$ -96° in H_2O).

Found: C, 51·7; H, 8·2; N, 6·9.

Calc. for $C_9H_{17}O_4N$: C, 53·2; H, 8·4; N, 6·9%.

The mother liquors were concentrated to a syrup (XII) (3·5 g.).

(iii) N-Acetylation of the crystalline N-isopropylidene derivative. A solution of the crystalline derivative (XI) (4·0 g.) in water (100 ml.) was acidified and N-acetylated in exactly the same way as for the α anomer. The product was recrystallised from acetone to give methyl 3-acetamido-

3-deoxy-β-D-arabofuranoside (VII) (3·41 g., 84% of theoretical), m.p. 156°, identical with the product isolated from the ammonolysis of the 'syrupy' methyl 2:3-anhydro-D-lyxofuranoside.

(iv) Investigation of the residual syrup (XII).

After removal of the crystalline N-isopropylidene derivative (XI) from the ammonolysis product, the residual material (3·5 g.) was N-acetylated in the usual way. The syrupy product (3·4 g., 96% of theoretical) in dry acetone (50 ml.) was shaken with anhydrous copper sulphate (8 g.) and N sulphuric acid (0·08 ml.).[19] After 3 days the copper sulphate was removed and the solution neutralised with ammonium hydroxide. Evaporation gave a non-reducing syrup (3·0 g., $[\alpha]_D^{22}$ -48° (c, 1·5 in H_2O)), which was fractionated between chloroform and water as in a previous experiment. The aqueous fraction gave a syrup (2·65 g., 88·5% of theoretical) which was distilled, b.p. 170-175°/0·1 mm., (0·94 g.), $[\alpha]_D^{21}$ -46° (c, 1·0 in H_2O) (c.f. methyl 3-acetamido-3-deoxy-β-D-arabofuranoside, $[\alpha]_D$ -120° in H_2O). The syrup was non-reducing to Fehling's solution before, and reducing after, hydrolysis with hot dilute hydrochloric acid, and gave a negative test for the presence of the isopropylidene group. Chromatographic examination (see p.31) showed the presence of two major components, R_G 0·60 and R_G 0·70 (c.f. R_G of methyl 3-acetamido-3-deoxy-β-D-arabofuranoside 0·64). A solution of this syrup in ethanol-light

petroleum deposited crystals (60 mg.) which had m.p. 212-214°, $[\alpha]_D^{26}$ -64° (c, 0·9 in H_2O), and R_G 0·60.
Found: C, 46·3 ; H, 7·3 ; N, 5·7 .
$C_8H_{15}O_5N$ requires C, 46·8 ; H, 7·4 ; N, 6·8 %.
– Methyl 2-acetamido-2-deoxy-β-D-xylofuranoside.

The chloroform fraction yielded a dark brown syrup (70 mg., 2·3% of theoretical), which was non-reducing to Fehling's solution and gave a faint positive test for the presence of the isopropylidene group.

Methylation of methyl 3-acetamido-3-deoxy-β-D-arabofuranoside.

Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside (VII) (2·0 g.) in methanol (15 ml.) was methylated at 40° in the usual way with methyl iodide (23 ml.) and silver oxide (20 g.). After 3 methylations, a solution of the crystalline product in acetone-light petroleum deposited crystals (0.90 g.), m.p. 154-157°. A cooled solution of these in acetone gave crystals 'A' (580 mg.), and on addition of light petroleum further crystals 'B' (280 mg.) were isolated.

Examination of 'A'. These crystals were non-reducing to Fehling's solution, and, after recrystallisation from acetone as prisms, had m.p. 157°, $[\alpha]_D^{24}$ -118° (c, 0.7 in H_2O) and mixed m.p. determinations gave a depression with starting material, fraction 'B', and authentic methyl

3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside. Chromatographic examination (see p. 31) showed the presence of only one component R_G 0·77.

Found: ~~C, 49·7~~ ; ~~H, 7·9~~ ; N, ~~5·8~~ 6·3 ; OMe, 28.3.

$C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4; OMe, 28·3%.

The crystals (50 mg.) were tosylated in the usual way and the toluene-p-sulphonyl ester (mp. 85-86° $[\alpha]_D$ −69° (c 0.9 in $CHCl_3$)) isolated (55 mg.) gave a positive test for the presence of a primary toluene-p-sulphonyloxy group,[63] thus identifying the crystals as methyl 3-acetamido-3-deoxy-2-O-methyl-β-D-arabofuranoside.

Examination of 'B'. These crystals were non-reducing to Fehling's solution and, after recrystallisation from acetone-light petroleum as needles, had m.p. 156-160°, and mixed m.p. determinations showed them to be different from starting material, fraction 'A', and authentic methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside. Chromatographic analysis gave two spots having R_G 0·92 and R_G 0·78 (c.f. R_G values for authentic methyl 3-acetamido-3-deoxy-2,5-di-O-methyl-β-D-arabofuranoside (0·92) and Fraction 'A' (0·77).

Found: C, 51·0; H, 8·5; N, 5·0; OMe, 36·2.

$C_{10}H_{19}O_5N$ requires C, 51·5; H, 8·2; N, 6·0; OMe, 39·9.

$C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4; OMe, 28·3%.

($C_{10}H_{19}O_5N$ = Methyl 3-acetamido-3-deoxy-2,5-di-O-methyl-β-D-arabofuranoside.

$C_9H_{17}O_5N$ = Methyl 3-acetamido-3-deoxy-mono-O-methyl-β-D-furanoside).

Methylation of the residues. The material left after the separation of these two fractions (1·1 g., OMe, 24·9%) was remethylated three times as before. The product (1·0 g., OMe 36·5%) was extracted with cold acetone (2 ml.) and the extract treated with light petroleum. Needle shaped crystals of methyl 3-acetamido-3-deoxy-2,5-di-O-methyl-β-D-arabofuranoside were deposited (0·51 g.) which after recrystallisation from the same solvents had m.p. 174-175°, $[\alpha]_D^{23}$ -107° (c, 0·8 in H_2O), and R_G 0·92. (P31)

Found: C, 51·6 ; H, 8·1 ; N, 5·9 ; OMe, 39.4.

$C_{10}H_{19}O_5N$ requires C, 51·5; H, 8·2; N, 6·0; OMe, 39·9%.

The Ammonolysis of Methyl 2:3-Anhydro-5-O-methyl-α-D-lyxofuranoside.

(i) Ammonolysis. Methyl 2:3-anhydro-5-O-methyl-α-D-lyxofuranoside (9·1 g.) in methanolic ammonia (500 ml.) was heated in an autoclave at 120° for 48 hours. The resulting brown solution was filtered and treated with charcoal at room temperature. Removal of the solvent gave a brown syrup which was freed from ammonia by solution in ethanol and concentration to a syrup (9·51 g., 94% of theoretical).

(ii) Separation of a crystalline N-isopropylidene derivative. The crude ammonolysis product (9·51 g.) was dissolved in hot acetone (50 ml.) and the cooled solution deposited crystals, shown to be methyl 3-amino-3-deoxy-N-isopropylidene-5-O-methyl-α-D-arabofuranoside (XIII) (8·34 g., 72% of theoretical). After several recrystallisations from acetone, they had m.p. 115-125°, $[\alpha]_D^{22}$ +106° (c, 0·9 in H_2O).

Found: C, 54·3; H, 9·4; N, 6·4.

$C_{10}H_{19}O_4N$ requires C, 55·3; H, 8·8; N, 6·5.

$C_7H_{15}O_4N$ requires C, 47·4; H, 8·5; N, 7·9%.

($C_7H_{15}O_4N$ = Methyl 3-amino-3-deoxy-5-O-methyl-α-D-arabofuranoside).

Concentration of the mother liquors gave a dark brown syrup (2·82 g., $[\alpha]_D$ +65° (c, 0·7 in H_2O)), which was distilled b.p. 100° (bath temperature)/0·03 mm., and this

syrup (520 mg.) yielded a further crop of the above crystals (80 mg.).

(iii) N-Acetylation of the crystalline N-isopropylidene derivative. The crystalline N-isopropylidene derivative (XIII) (5·81 g.) in water (150 ml.) was acidified with 2N hydrochloric acid and N-acetylated in the usual way. The syrupy product (5·80 g., 99% of theoretical) partly crystallised but the crystals, m.p. 43-45°, could not be recrystallised. Distillation gave a colourless syrup (4·64 g.) b.p. 140° (bath temperature)/0·05 mm., which crystallised. This product could not be recrystallised but the crystals could be freed from syrup by washing with ether. These hygroscopic crystals, methyl 3-acetamido-3-deoxy-5-O-methyl-α-D-arabofuranoside, (3·87 g.), had m.p. 60-62°, $[\alpha]_D^{21}$ +119° (c, 0.9 in H_2O), and R_G 0.88. (p. 31)

Found: C, 48·2 ; H, 7·6 ; N, 5·8 ; OMe, 27.8.

$C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4; OMe, 28·3%.

(iv) Hydrolysis of the crystalline N-isopropylidene derivative. The N-isopropylidene derivative (XIII) (227 mg.) in 2N hydrochloric acid (15 ml.) was heated at 100° until the rotation of the solution reached a constant value.

Time (min.)	α_D (1 dm.)
0	+1·97°
10	+0·70°
25	+0·33°
50	+0·26°
70	+0·25°

The hydrolysis solution was evaporated to dryness but no crystalline hydrochloride could be isolated.

Methylation of methyl 3-acetamido-3-deoxy-5-O-methyl-α-D-arabofuranoside.

Methyl 3-acetamido-3-deoxy-5-O-methyl-α-D-arabofuranoside (1·0 g.) was methylated four times with methyl iodide (23 ml.) and silver oxide (20 g.) in the usual way. The product (1·07 g.) was a mobile syrup and had n_D^{22} 1·4548. Distillation gave two fractions (1) 310 mg., b.p. 80° (bath temperature)/0·02 mm., the nature of which is being investigated, and (2) 520 mg., b.p. 100° (bath temperature)/0·02 mm., which crystallised completely on standing. The crystals from the latter fraction were isolated by washing with light petroleum and were identical with the methyl 3-acetamido-3-deoxy-2,5-di-O-methyl-α-D-arabofuranoside previously prepared. They had m.p. and mixed m.p. with authentic material 57-59°, $[\alpha]_D^{21}$ +129° (c, 0·9 in H_2O), and R_G 0·97. (p.31)

Found: OMe, 39·1.

$C_{10}H_{19}O_5N$ requires OMe, 39·9%.

The Ammonolysis of 'Syrupy' Methyl 2:3-Anhydro-5-O-methyl -αβ-D-lyxofuranoside.

(i) Ammonolysis. 'Syrupy' methyl 2:3-anhydro-5-O-methyl-αβ-D-lyxofuranoside (1·32 g.) in methanolic ammonia (60 ml.) was heated in a sealed tube at 120° for 48 hours. The product was a brown syrup (1·40 g., 96% of theoretical). This on distillation gave a colourless syrup (XIV) (0·76 g.), b.p. 110-120° (bath temperature)/0·02 mm., $[\alpha]_D^{18}$ -42° (c, 1·4 in EtOH).

(ii) N-Acetylation. The distilled product (XIV) (535 mg.) was N-acetylated in the usual way and the product (605 mg., 91% of theoretical) was a syrup which on solution in acetone and addition of light petroleum deposited crystals (125 mg.). After recrystallisation from the same solvents, these had m.p. 161-162°, $[\alpha]_D^{18}$ -111° (c, 0·4 in H_2O), and were shown to be identical with the methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside subsequently prepared.

Found: C, 49·6; H, 7·8; N, 6·3.

$C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4%.

No further crystals could be obtained from the mother liquors.

The Ammonolysis of Methyl 2:3-Anhydro-5-O-methyl-β-D-lyxofuranoside.

(i) Ammonolysis. Methyl 2:3-anhydro-5-O-methyl-β-D-lyxofuranoside (6·2 g.) was treated with methanolic ammonia (500 ml.) in exactly the same way as the α anomer. The product was a brown syrup (6·17 g., 90% of theoretical).

(ii) Attempted preparation of an N-isopropylidene derivative. The crude ammonolysis product was dissolved in hot acetone as in previous experiments but no crystalline product separated. Removal of the acetone followed by distillation gave a colourless syrup (XV) (5·64 g., 74% of theoretical), b.p. 84-86°/0·03 mm.

(iii) N-Acetylation. The distilled mixture (XV) in water (150 ml.) was acidified and N-acetylated in the usual way. The product (5·44 g., 96% of theoretical) on solution in acetone and addition of light petroleum gave crystals (2·66 g., 49% of the N-acetylated product) shown to be methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside. After recrystallisation from acetone-light petroleum, the crystals had m.p. 161-162°, $[\alpha]_D^{23}$ -114° (c, 1·2 in H_2O), and R_G 0·81. (p.31)

Found: C, 49.5 ; H, 7.8 ; N, 6.8 ; OMe, 28.1.

$C_9H_{17}O_5N$ requires C, 49·3; H, 7·8; N, 6·4; OMe, 28·3%.

Evaporation of the mother liquors gave a brown syrup which distilled as a syrup (71 mg.), $[\alpha]_D^{20}$ +30° (c, 0·7

in H_2O). An aqueous extract of the distillation residues gave, on treatment with charcoal and evaporation, a syrup (80 mg.), $[\alpha]_D^{23}$ +18° (c, 0·8 in H_2O).

Methylation of methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside.

Methyl 3-acetamido-3-deoxy-5-O-methyl-β-D-arabofuranoside (1·0 g.) in methanol (5 ml.) was methylated four times in the usual way. The product (1·0 g.) on solution in acetone and addition of light petroleum deposited crystals (750 mg.) identical with the methyl 3-acetamido-3-deoxy-2,5-di-O-methyl-β-D-arabofuranoside previously prepared. They had m.p. and mixed m.p. with authentic material 174-175°, $[\alpha]_D^{23}$ -107° (c, 1·1 in H_2O), and RG 0.92. (p.31)

Found: OMe, 40·1.

$C_{10}H_{19}O_5N$ requires OMe, 39·9%.

PART II

Syntheses of Glucosamine 6-Phosphate

PART II

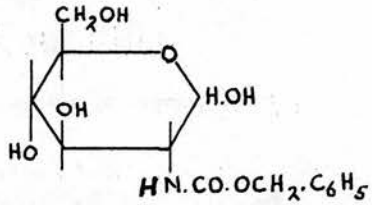
SYNTHESES OF GLUCOSAMINE 6-PHOSPHATE

Discussion

In order to synthesise authentic glucosamine 6-phosphate and to avoid the danger of contamination with derivatives carrying phosphate residues on other carbon atoms in the sugar chain, it is necessary, firstly to protect the amino group and all the secondary hydroxyl groups in the molecule before phosphorylation, and secondly to choose substituents for these groups that can be removed without affecting the phosphate residue on C_6. It is also highly desirable to obtain crystalline derivatives at each stage in the synthesis. The well-known paper chromatographic method of analysis of syrupy materials is notoriously difficult to apply to amino sugars owing to the diffuse nature of the spots obtained and to the lack of satisfactory detecting reagents.(10)

In general three different types of substituents have been used to protect the amino group in syntheses of glucosamine derivatives. Condensation of aromatic aldehydes with this group gives a Schiff's base type of compound.(43) In particular, Bergmann and Zervas(44) used the derivative prepared from p-methoxy-benzaldehyde to prepare 1,3,4,6-tetra-O-acetyl-glucosamine. Methyl glucosaminides have

frequently been prepared[12] by first blocking the amino group with the carbobenzyloxy residue.



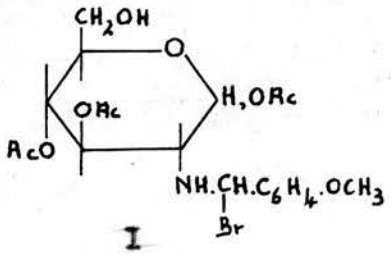
N-Carbobenzyloxy-D-glucosamine

Acetylation has been used successfully by Jeanloz[40,45,46] in the preparation of partly methylated derivatives of glucosamine.

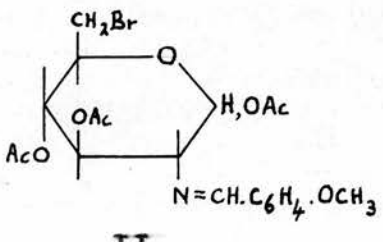
In a preliminary investigation, it was decided to use N-p-methoxybenzylidene-glucosamine as starting material. (Formulae of the intermediate compounds prepared during this synthesis will be found on p.87). This compound was readily obtained in a crystalline form and in excellent yield by the method of Bergmann and Zervas.[44] The blocking of the primary hydroxyl group with a trityl residue and the remaining hydroxyl groups with acetyl residues was conveniently carried out in one operation in a manner similar to that used by Reynolds and Evans.[38] The resulting material was amorphous and separation of a single anomer was achieved once only on a very small scale. The crystalline product isolated was analytically pure and its high positive specific rotation suggested that it was the α-anomer of 1,3,4-tri-O-acetyl-N-p-methoxybenzylidene-6-O-triphenylmethyl-D-glucosamine. The amorphous material

had an acetyl content 1% lower than the theoretical.

The preferential removal of the trityl group from this latter material was attempted by various methods. Treatment with 60% acetic acid at 100°[40] gave a syrupy product analysis of which indicated that partial hydrolysis of the p-methoxybenzylidene group had also occurred. Removal of the trityl residue with hydrogen bromide in glacial acetic acid[47] gave, in a preliminary experiment, a syrup which analysed correctly for the expected triacetyl Schiff's base. In the large scale preparation however, the syrup was contaminated with tritanol and several attempts were made to remove this. Separation on an alumina column[48] was effective in separating the tritanol but the basic nature of the alumina caused partial de-acetylation of the sugar derivative. Finally, the tritanol was removed by exhaustive extraction with light petroleum, but the residual syrup was shown to contain a small amount of bromine, presumably due either to addition of hydrogen bromide at the double-bond of the Schiff's base (I) or to partial conversion to the 6-bromo-6-deoxy derivative (II).[88]



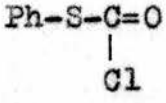
I



II

Phosphorylation was carried out[26] with diphenyl-phosphorochloridate prepared by the method of Foster, Overend, and Stacey.[42] Elemental analysis of the syrupy product was in agreement with the composition of the expected diphenyl phosphate derivative. Attempted removal of the phenyl groups by hydrogenolysis[26] again gave a syrup, analysis of which indicated that only partial removal of the phenyl groups had taken place. De-acetylation with potassium methoxide and hydrolysis of the p-methoxybenzylidene group were attempted, but no recognisable product could be isolated. It seemed likely that the double bond of the Schiff's base had been saturated under the conditions used for the hydrogenolysis of the phenyl groups. In view of this and of the failure to obtain crystalline derivatives in the later stages of the synthesis, this route was abandoned.

Search was then made for other substituents of the amino group. Phenyl thiocarbonyl chloride (III) has been



III

used to protect amino groups in amino acids,[49] but no crystalline derivative of glucosamine could be prepared (see p. 127). Experiments were carried out to determine the conditions necessary for the complete hydrolysis of N-acetyl-glucosamine and β-penta-acetyl-glucosamine (p. 128).

Since these did not appear to be sufficiently drastic to cause hydrolysis of the phosphate group, a second synthesis of glucosamine 6-phosphate was planned in which acetyl groups were used to protect the amino group as well as the secondary hydroxyl groups. (For formulae of the compounds prepared during this synthesis, see p.94).

Instead of the older method of preparation of N-acetyl-glucosamine with silver acetate and acetic anhydride,[50] that reported by Roseman and Ludowieg[36] was used. Acetylation was carried out in a cold aqueous solution of acetic anhydride, neutrality being maintained by the addition of the carbonate form of an anion-exchange resin. Unchanged glucosamine was removed from the reaction mixture by passage through a column of cation-exchange resin and crystalline N-acetyl-α-D-glucosamine was isolated in good yield from the eluate. The unchanged glucosamine was recovered as the hydrochloride from the resin column by elution with N hydrochloric acid.

Simultaneous tritylation and acetylation is usually carried out at 100° [38] but preliminary experiments in this synthesis were done at 40° in an attempt to retain the α-configuration and to reduce decomposition. An amorphous substance was obtained which on fractional crystallisation gave two products. The first of these was not very soluble in chloroform and had a very high melting point. Elemental analysis and trityl content[41] showed this to be a tri-

acetyl-ditrityl-glucosamine. It was non-reducing to Fehling's solution, whereas compounds with the reducing group blocked by an acetyl residue were reducing. This suggested that the compound was 3,4-di-O-acetyl-N-acetyl-1,6-di-O-triphenylmethyl-D-glucosamine. Confirmation of this was obtained by removal of the trityl residues. A tri-acetyl-glucosamine was isolated which showed mutarotation in aqueous solution, and which proved to be identical with the tri-acetate produced by detritylation and partial de-acetylation of the authentic 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine prepared in later experiments. The negative specific rotation (-29°) of the ditrityl derivative suggested that it had the β-configuration. Steric factors may hinder the formation of the other anomer. The reason for the formation of this ditrityl derivative is undoubtedly the relative insolubility of N-acetyl-glucosamine at the reaction temperature with the resulting high proportion of trityl chloride to sugar derivative in the solution. The formation 1,6-ditrityl ethers of D-xylose, D-ribose, and L-arabinose has been reported in experiments where a high proportion of trityl chloride was present.(48)

The second product isolated from the fractional crystallisation, after recrystallisation from chloroform-light petroleum, showed, on analysis, the presence of solvent of crystallisation. Complete removal of the solvent gave a crystalline material which analysed correctly for 1,3,4-

tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-D-glucosamine. The solvated product, from the analytical figures and from quantitative desolvation, was shown to contain more than one solvent. The presence of chlorine indicated that chloroform is present but in insufficient quantity to account for all the solvent. The other solvent may be light petroleum or may be due to ethanol, present as impurity in the chloroform.

In view of the results of this preliminary experiment, it was decided to revert to tritylation at 100° followed by acetylation without cooling.(38) Three crystalline fractions were obtained. The first fraction was deposited on cooling a hot carbon tetrachloride solution of the crude material. After one recrystallisation it was isolated in 25% yield with solvent of crystallisation. Removal of the solvent gave crystals of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine which was characterised by melting point, analysis, and specific rotation. The solvated material was shown to contain four thirds of a molecule of carbon tetrachloride to every molecule of sugar. Recrystallisation from other solvents appeared to give other solvates but these were not examined. This phenomenon of solvent of crystallisation is seldom encountered in non-ionic sugar derivatives. Trityl ethers, however, appear to be more liable than most derivatives to behave in this way. For

example, the preparation of the ethyl alcoholate of dityrityl-dulcitol has been reported,[51] and Edington, Hirst and Percival[52] have isolated methyl tribenzoyl-6-trityl-α-D-mannopyranoside with one molecule of pyridine, one molecule of acetone, or three quarters of a molecule of chloroform of crystallisation.

After removal of the solvent from the mother liquors, the residual syrup was dissolved in warm chloroform. Addition of an equal volume of ether and cooling caused the deposition of crystals of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-D-glucosamine as a mixed solvate (5% of theoretical). 3,4-Di-O-acetyl-N-acetyl-1,6-di-O-triphenyl-methyl-D-glucosamine was isolated in 2% yield on the addition of light petroleum to the residual solution.

It was hoped to increase the yield of the β-anomer of the tetra-acetyl-6-trityl-glucosamine by suitable treatment of the mother liquors. With this in view, the crystalline α-anomer was heated with acetic anhydride in pyridine for varying lengths of time. Unchanged starting material was invariably recovered, however, indicating that no anomerisation had taken place. This failure to achieve partial anomerisation of the α-form has been met previously in the methyl glycosides of glucosamine[12,53] and of 3-amino-glucose.[54]

Several methods were investigated for the removal of

the trityl residues and difficulties in isolation were encountered due to the unusual relative solubility of the resulting acetates in chloroform and water. When an acetic acid solution of the ditrityl ether was treated with hydrogen bromide in acetic acid,[47] extraction of an aqueous solution of the product with chloroform failed to give any significant yield of the tri-acetate, due to its preferential solubility in water. An attempt was made to utilise this relative insolubility in chloroform by treating the ditrityl ether with hydrogen chloride in this solvent[39] but no crystalline acetate was deposited. Hydrogenolysis of the trityl groups[39] was attempted but, possibly due to poisoning of the catalyst, no cleavage appeared to occur. Use of 60% acetic acid at 100°, however, gave crystalline 3,4-di-O-acetyl-N-acetyl-α-D-glucosamine, identified by elemental analysis and by the fact that it showed mutarotation in aqueous solution.

Treatment of the tetra-acetyl-6-trityl-α-D-glucosamine according to all the methods used for the ditrityl ether failed to yield a crystalline product. When the β-anomer of the tetra-acetyl-6-trityl derivative was heated at 100° with 60% acetic acid, a moderate yield of a crystalline tri-acetate was obtained. This was identical with the tri-acetate isolated from the detritylation of tri-acetyl-1,6-ditrityl-glucosamine. Penta-acetyl-β-D-glucosamine was subsequently shown to undergo partial hydrolysis under

these conditions, the recovery of starting material being only 33%. This method was clearly unsuitable for the detritylation of compounds with an acetyl group at C_1 and possibly for acetates in general, although it has been used successfully by Jeanloz in the detritylation of glucosamine derivatives containing an N-acetyl group[40,46] and O-benzoyl groups.[46]

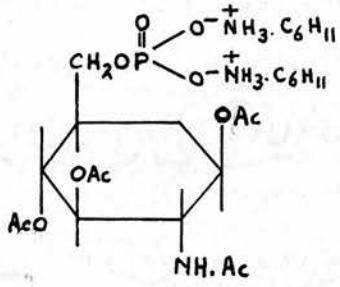
Preliminary experiments on the detritylation of the solvate of tetra-acetyl-6-trityl-β-D-glucosamine with hydrogen bromide in acetic acid gave negligible yields of product on chloroform extraction of the aqueous solution. Investigation showed that, whereas 1,2,3,4-tetra-acetyl-β-D-glucose may be isolated readily by chloroform extraction of an aqueous solution,[38] the analogous glucosamine derivative was preferentially soluble in water, the partition ratio between chloroform and water being 1:3. This difficulty was overcome by exhaustive chloroform extraction and elimination of the usual water washing of the extracts. The crystalline β-tetra-acetate was obtained in good yield, the reaction being apparently unaffected by the presence of the carbon tetrachloride in the starting material.

In view of the ease of preparation of the β-form of the trityl ether, as compared with the α-anomer and also the ready preparation of crystalline 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine, it was decided to continue the synthesis with this material. But before proceeding with

the synthesis the structure of this compound was confirmed. It analysed correctly for a tetra-acetyl derivative of glucosamine and failed to show any mutarotation in aqueous solution indicating that C_1 was blocked by an acetyl residue. The principal danger was the possibility of acetyl migration during the hydrolysis of the trityl group.(55) Conversion of 1,2,3,4-tetra-acetyl-β-D-glucose to the 1,2,3,6-tetra-acetate has been shown to occur readily in alkaline solution and even in neutral solutions contained in a soft-glass vessel.(56) If the tetra-acetyl-glucosamine has the postulated structure and no migration of acetyl groups has occurred, then tritylation should give the parent trityl ether. Some difficulty was experienced in carrying out this reaction at room temperature but reaction at 50° gave the desired product. The analogous 1,2,3,4-tetra-acetyl-β-D-glucose was also shown to be resistant to tritylation at room temperature. Final proof that the primary hydroxyl group was free was obtained by tosylation. A crystalline mono-toluene-p-sulphonyl-derivative was isolated which gave a positive test for the presence of a primary toluene-p-sulphonyloxy group when treated under the standard conditions with acetone and sodium iodide.(63)

Phosphorylation of 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine in pyridine solution with a slight excess of diphenyl phosphorochloridate gave a crystalline diphenyl phosphate. Removal of the phenyl groups by a platinum

catalysed hydrogenolysis proceeded smoothly at room temperature in ethanolic solution. In experiments on the large scale, the time required for the uptake of hydrogen to reach completion varied considerably, presumably due to differences in the physical state of the catalyst, and the uptake was always a little below that required by theory. Removal of the solvent (at low temperature to prevent autohydrolysis) gave a syrup which crystallised readily from ethanol. Although one attempt to prepare a crystalline cyclohexylamine salt (IV) was apparently successful, all



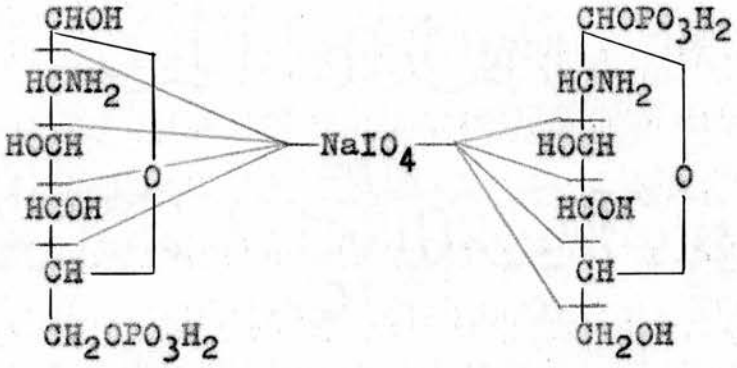
IV

subsequent attempts failed. No adequate explanation for this can be put forward.

A preliminary hydrolysis of the acetyl groups with N hydrochloric acid to constant rotation failed to give either a crystalline glucosamine phosphoric acid or a crystalline cyclohexylamine salt. The hydrolysis was repeated and the barium salt of the sugar phosphate isolated by neutralisation of the hydrolysate to pH 7 with barium hydroxide. Although the product, after purification by several

reprecipitations from water, had a theoretical phosphorus content, the carbon and hydrogen contents were too high. This may be due either to incomplete de-acetylation or to the addition of insufficient barium hydroxide to convert all the phosphoric acid ester to the barium salt. In view of this result hydrolysis for three hours in N sulphuric acid was carried out. It was observed that very little change in rotation occurred after the first 30 minutes. The solution was neutralised by shaking overnight with finely powdered barium carbonate. Removal of the insoluble barium salts and addition of ethanol gave a product which after drying was not readily soluble in water. This difficulty was overcome by the addition of a few drops of dilute hydrochloric acid. This material after reprecipitation had a theoretical organic phosphorus content and a negligible amount of inorganic phosphate, but, although the carbon content was nearer the theoretical, this value and that for hydrogen were still too high. The hydrolysis with sulphuric acid was repeated and the sugar phosphate isolated as the free phosphoric acid derivative by careful neutralisation of the sulphuric acid with barium hydroxide. Since the barium salt of the sugar phosphate is soluble in water, it was possible to ensure that a negligible quantity of this derivative was formed as the presence of barium ions could be detected with the very sensitive reagent rhodizonic acid.[58] After removal of the insoluble barium sulphate,

and addition of ethanol to the concentrated filtrate, crystals were deposited on cooling. These analysed correctly for the monohydrate of a glucosamine monophosphate and gave a single spot on a paper chromatogram. In view of the established structure of the crystalline 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine and the isolation of crystalline derivatives at each subsequent stage, there is little doubt that this material is D-glucosamine 6-(dihydrogen phosphate). This was further confirmed by the fact that it consumed 4 moles of periodate for every mol. of sugar phosphate indicating that the phosphate must be situated at either C_1 or C_6.



The absence of formaldehyde[59] in a periodate oxidised solution of the phosphate showed the absence of an unsubstituted primary hydroxyl group. Furthermore Brown[33] reports that glucosamine 1-phosphate is readily hydrolysed in acid solution. Glucosamine 6-phosphate was shown to be considerably more resistant to acid hydrolysis than even glucose 6-phosphate. The latter compound was 50% hydrolysed in N hydrochloric acid at 100° in 23 hours[60]

whereas the glucosamine derivative, under exactly the same conditions, required 73 hours. The crystalline phosphate had $[\alpha]_D^{21}$ +54° (calc. for the anhydrous compound $[\alpha]_D^{21}$ +57·5°) whereas Brown[33] records $[\alpha]_D^{24}$ +48·5° for the dipolar ion.

The identity of the barium salt isolated is not so certain. It too consumed the theoretical amount of periodate for a 6-phosphate and gave no formaldehyde on periodate oxidation.

The acidity of a 7% aqueous solution of the tetra-acetyl 6-phosphoric acid derivative was found to be sufficient to effect hydrolysis of the acetyl groups. After heating this solution at 100° for 30 hours, a small quantity of a crystalline dihydrate of glucosamine 6-(dihydrogen phosphate) was isolated.

The estimation of the glucosamine content of the glucosamine 6-phosphate by the colorimetric method of Elson and Morgan[61] as modified by Belcher, Nutten, and Sambrook[62] was shown to be unsatisfactory, the solutions obtained being browner than those given by the glucosamine controls and the intensity indicating a glucosamine content of only 59% of the theoretical if the solutions were allowed to stand after adjustment to PH 7. But if the estimations were carried out on fresh solutions then 96-98% of the theoretical quantity of glucosamine was given.

While the work on an unambiguous chemical synthesis of glucosamine 6-phosphate was proceeding, the possibility of the preparation of this compound by direct phosphorylation was investigated. The preparation of glucose 6-phosphate has been reported by Seegmiller and Horecker[28] by treatment of glucose with 'tetraphosphoric acid' and by Viscontini and Olivier[29] using metaphosphoric acid. As the latter reagent gave a purer product in better yield, and is stated to react preferentially with primary hydroxyl groups to the exclusion of secondary hydroxyl and amino groups, it was decided to investigate its reaction with glucosamine. In the phosphorylation of glucose, however, the reducing group also appeared to be phosphorylated, but the reducing power was restored by mild acid hydrolysis. In order to reduce caramelisation of the glucose, methyl cyanide was added during the phosphorylation.

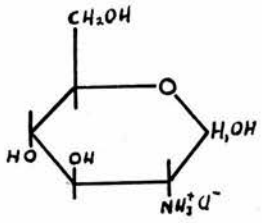
When the phosphorylation of glucosamine was carried out under these conditions, it was found necessary to increase the reaction time from 40 minutes to 2·5 hours and then to re-treat the product for a further 1·5 hours in order to raise the phosphorus content to the same value as that obtained in the glucose preparation.

Hydrolysis of the product with N hydrochloric acid at 100° was carried out. Variation in the time of hydrolysis had no apparent effect on the composition of the hydrolysate. Chromatographic examination of this showed the presence of

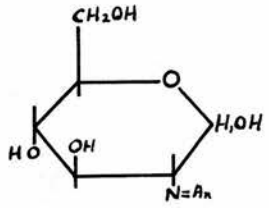
glucosamine hydrochloride and a reducing sugar phosphate.

Attempted separation on a column of anion-exchange resin (Deacidite F)[29] led to extensive decomposition of the phosphate. Products with very low phosphorus and high nitrogen contents were isolated. Separation as the amorphous barium 6-phosphate met with more success but considerable difficulty was experienced in purifying the material with consequent serious reduction in the yield. Although apparently chromatographically pure and analysing correctly for the barium salt of a glucosamine mono-phosphate, it oxidised considerably more slowly with periodate than the authentically synthesised material. The consumption of periodate was 70% of the theoretical after 22 hours compared with 94% of theoretical for the barium salt isolated by the synthetic method. Furthermore formaldehyde was produced during the reaction in quantity corresponding to the presence of about 35% of a derivative carrying a free primary hydroxyl group. It would appear therefore that the product obtained contained only 60-70% of the desired glucosamine 6-phosphate and that the method, particularly in view of the poor yields, is unsuitable for the preparation of this derivative.

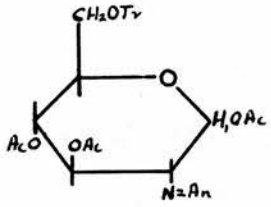
The Synthesis of D-Glucosamine 6-Phosphate (Method I)



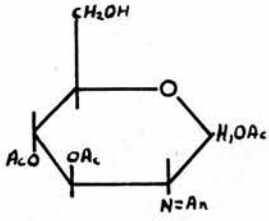
D-Glucosamine Hydrochloride



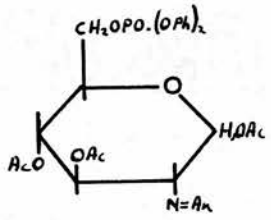
N-Anisal-D-glucosamine



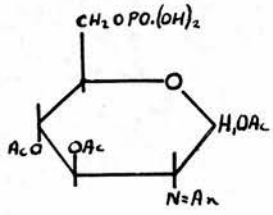
1,3,4-Tri-acetyl-N-anisal-6-trityl-D-glucosamine



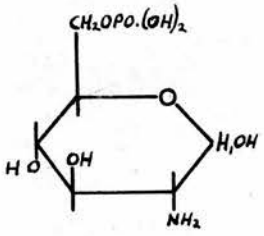
1,3,4-Tri-acetyl-N-anisal-D-glucosamine



1,3,4-Tri-acetyl-N-anisal-D-glucosamine 6-(diphenyl phosphate)



1,3,4-Tri-acetyl-N-anisal-D-glucosamine 6-(dihydrogen phosphate)



D-Glucosamine 6-(dihydrogen phosphate)

N-p-Methoxybenzylidene-D-glucosamine.

Preliminary Experiments. (a) D-Glucosamine hydrochloride (1·0 g.) in N sodium hydroxide solution (4·7 ml.) was shaken with twice-distilled anisaldehyde (0·64 g.) for 2 hours.(44) The crystalline solid which separated was washed with ice-water (1 ml.) and dried. These crystals (1·35 g., 98% of theoretical) after recrystallisation from ethanol had m.p. 176-177° $[\alpha]_D^{20}$ +44° (c, 0·2 in EtOH). (Bergmann and Zervas(44) record yield 86%, m.p. 166° for N-p-methoxybenzylidene-D-glucosamine).

(b) D-Glucosamine hydrochloride (1·0 g.) and sodium bicarbonate (0·55 g.) in water (12 ml.) were shaken with anisaldehyde (0·80 g.) for 2 hours.(70) The resulting mixture deposited, on cooling, crystals of N-p-methoxybenzylidene-D-glucosamine (0·65 g., 47% of theoretical), m.p. 171-173°.

Large Scale Preparation. D-Glucosamine hydrochloride (20·0 g.) in N sodium hydroxide solution (94 ml.) was shaken for 2 hours with anisaldehyde (12·8 g.). Filtration and washing gave the desired product (25·9 g., 94% of theoretical), m.p. 172-174°.

1,3,4-Tri-O-acetyl-N-p-methoxybenzylidene-6-O-triphenylmethyl-D-glucosamine.

Preliminary Experiment. N-p-Methoxybenzylidene-D-glucosamine (1·0 g.) in dry pyridine (5 ml.) was heated with triphenylchloromethane (0·91 g.) and 'Drierite' (0·5 g.) at 50° for 4 hours. Without cooling, acetic anhydride (8·0 ml.) was added and the mixture allowed to stand overnight at room temperature.(38) The filtered solution was poured into vigorously stirred ice-water (600 ml.) and the precipitate which formed was washed with water till free from pyridine and dried. The amorphous powder (1·43 g., 64% of theoretical) had $[\alpha]_D^{15}$ +37° (c, 1·0 in $CHCl_3$).

Found: CH_3CO, 18·4.

Calc. for $C_{39}H_{39}O_9N$: CH_3CO, 19·4%.

Slow evaporation of an ethanolic solution of this powder gave a crystalline product (300 mg., 13% of theoretical). On recrystallisation from ethanol the crystals had m.p. 178-179°, $[\alpha]_D^{15}$ +90° (c, 0·6 in $CHCl_3$).

Found: C, 70·2; H, 5·7; N, 2·2; CH_3CO, 19·1.

$C_{39}H_{39}O_9N$ requires C, 70·4; H, 5·9; N, 2·1; CH_3CO, 19·4%.

Large Scale Preparation. N-p-Methoxybenzylidene-D-glucosamine (21·0 g.), in dry pyridine (100 ml.) was treated with triphenylchloromethane (20·0 g.) and acetic anhydride (168 ml.) as in the preliminary experiment. The dried product, a white powder (35 g., 74% of theoretical), had $[\alpha]_D^{15}$ +29° (c, 1·1 in $CHCl_3$).

Found: CH_3CO, 18·5%.

All attempts to obtain a crystalline fraction failed.

1,3,4-Tri-O-acetyl-N-p-methoxybenzylidene-D-glucosamine.

Preliminary Experiments. (a) 1,3,4-Tri-O-acetyl-N-p-methoxybenzylidene-6-O-triphenylmethyl-D-glucosamine (1·3 g.) was dissolved in glacial acetic acid at 100°, water (16 ml.) was added dropwise, and the heating continued for 1 hour.(40) Water (80 ml.) was added to the cooled solution and the precipitated triphenylcarbinol removed. The aqueous filtrate was concentrated and the residue, freed from acetic acid by repeated addition of dry toluene and evaporation, was obtained as a syrup (0·80 g., 95% of theoretical).

Found: CH_3CO, 34.

Calc. for $C_{20}H_{25}O_9N$: CH_3CO, 30·5%.

Solution in ethanol and treatment with charcoal at reflux temperature gave, on filtration and evaporation, a syrup (0·62 g., 74% of theoretical), $[\alpha]_D^{15}$ +33° (c, 1·1 in $CHCl_3$).

Found: C, 52·4; H, 6·3; N, 3·8; CH_3CO, 37.

Calc. for $C_{20}H_{25}O_9N$: C, 56·7; H, 5·9; N, 3·3; CH_3CO, 30·5.

Calc. for $C_{12}H_{19}O_8N$: C, 47·2; H, 6·3; N, 4·6; CH_3CO, 42·3%.

($C_{20}H_{25}O_9N$ = 1,3,4-Tri-O-acetyl-N-p-methoxybenzylidene-D-glucosamine

$C_{12}H_{19}O_8N$ = 1,3,4-Tri-O-acetyl-D-glucosamine).

(b) The trityl ether (1·2 g.) in glacial acetic acid (5 ml.) at 10° was shaken with a cooled (10°) saturated solution of dry hydrogen bromide in acetic acid (1·2 ml.) for 60 seconds.[47] The precipitated triphenylbromomethane was removed and the filtrate poured immediately into ice-water (600 ml.). The precipitate of unchanged starting material and tritanol was removed and the filtrate extracted with chloroform (4 x 100 ml.). The combined chloroform extracts were washed with ice-water (2 x 40 ml.), dried, and concentrated to a syrup (0·37 g., 49% of theoretical), $[\alpha]_D^{15}$ +23° (c, 1·0 in $CHCl_3$).

Found: C, 56·3; H, 6·0; N, 2·9; CH_3CO, 29.

Calc. for $C_{20}H_{25}O_9N$: C, 56·7; H, 5·9; N,3·3; CH_3CO, 30·5%.

Large Scale Preparation. A cooled solution of the trityl ether (23 g.) was treated with hydrogen bromide and glacial acetic acid as before. A syrup (7·1 g., 48% of theoretical) was obtained.

Found: CH_3CO, 23; Br, 1·1.

Calc. for $C_{20}H_{25}O_9N$: CH_3CO, 30·5; Br, 0%.

Several attempts were made to purify this product. Addition of single and mixed solvents failed to give a crystalline product. Separation of the desired compound from impurities on a column (15 cm. x 2·75 cm.) of B.D.H. chromatographic grade alumina[48] was carried out. The column was washed with dry benzene and the crude product (1·14 g.) was applied in benzene solution. Elution with

benzene (18 l.) removed all the triphenylcarbinol (0·26 g.). Elution with ethanol (2 l.) gave on evaporation a syrup (0·56 g.).

Found: CH_3CO, 25%.

A qualitative test for the presence of trityl groups in this syrup was negative. Acetylation of the syrup (0·36 g.) in the usual way with acetic anhydride and pyridine gave a syrup (0·30 g.).

Found: CH_3CO, 36·2.

Calc. for $C_{22}H_{27}O_{10}N$: CH_3CO, 37·0%.

($C_{22}H_{27}O_{10}N$ = 1,3,4,6-Tetra-O-acetyl-N-p-methoxybenzylidene-D-glucosamine).

1,3,4-Tri-O-acetyl-N-p-methoxybenzylidene-D-glucosamine 6-(diphenyl phosphate).

Diphenyl phosphorochloridate (see p.110) (1·5 ml.) was added dropwise, with shaking and cooling, to a solution of 1,3,4-tri-O-acetyl-N-p-methoxybenzylidene-D-glucosamine (1·7 g.) in dry pyridine (6 ml.).[26] The reaction mixture was kept at 0° for 30 minutes and then left for 18 hours at 8°. The excess of acid chloride was decomposed by addition of a few drops of ice-water and the mixture after standing for 30 minutes was poured slowly into stirred ice-water. The precipitated product was washed several times with water and dried. The amorphous powder was purified by solution in ethanol, filtration, and concentration to a

syrup (1·4 g., 53% of theoretical) $[\alpha]_D^{15}$ +23° (c, 0·5 in $CHCl_3$).

Found: C, 58·3; H, 5·3; N, 2·2; P, 3·7; CH_3CO, 21.

Calc. for $C_{32}H_{34}O_{12}NP$: C, 58·6; H, 5·2; N, 2·1; P, 4·7; CH_3CO, 19·7%.

Attempted Preparation of 1,3,4-Tri-O-acetyl-N-p-methoxybenzylidene-D-glucosamine 6-(dihydrogen phosphate).

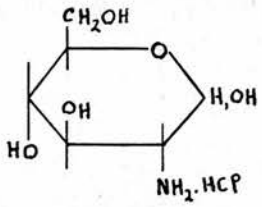
Hydrogen was bubbled through a stirred solution of 1,3,4-tri-O-acetyl-N-p-methoxybenzylidene-D-glucosamine 6-(diphenyl phosphate) (0·63 g.) in dry ethanol (15 ml.) for 5 hours in the presence of platinum (added as 60 mg. of Adam's platinum oxide).[26] After removal of the catalyst, the solution was concentrated to a syrup (0·57 g., 118% of theoretical), $[\alpha]_D^{16}$ +18° (c, 1·8 in $CHCl_3$).

Found: C, 57·2; H, 5·6; N, 2·4; P, 3·5; CH_3CO, 23.

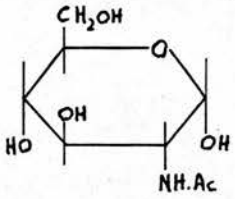
Calc. for $C_{20}H_{26}O_{12}NP$: C, 47·7; H, 5·2; N, 2·8; P, 6·2; CH_3CO, 25·6%.

De-acetylation of this crude product with potassium methoxide and hydrolysis of the N-p-methoxybenzylidene group with N hydrochloric acid at 100° were attempted but no recognisable product could be isolated.

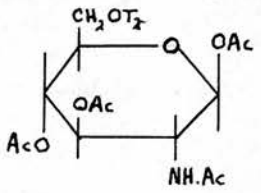
The Synthesis of D-Glucosamine 6-Phosphate (Method II).



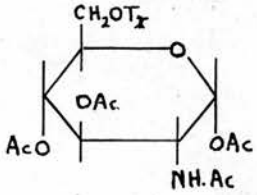
D-Glucosamine Hydrochloride



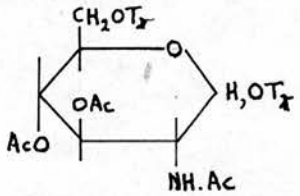
N-Acetyl-α-D-glucosamine



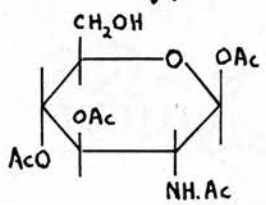
1,3,4-Tri-O-Acetyl-N-acetyl-6-O-trityl-β-D-glucosamine



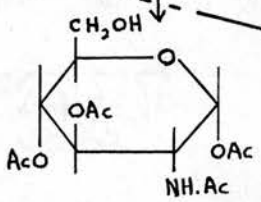
1,3,4-Tri-O-acetyl N-acetyl-6-O-trityl-α-D-glucosamine



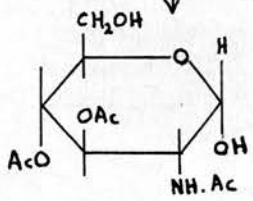
3,4-Di-O-acetyl-N-acetyl-1,6-di-O-trityl-D-glucosamine



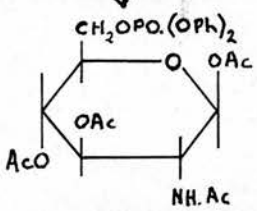
1,3,4-Tri-O-acetyl N-acetyl-β-D-glucosamine



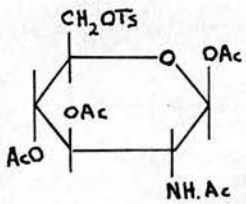
1,3,4-Tri-O-acetyl-N-acetyl-α-D-glucosamine



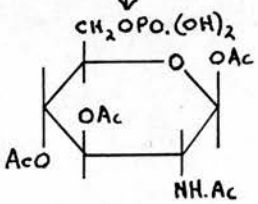
3,4-Di-O-acetyl-N-acetyl-α-D-glucosamine



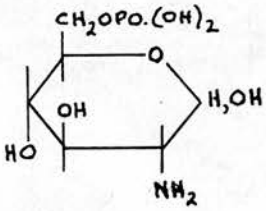
1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(Diphenyl phosphate)



1,3,4-Tri-O-acetyl-N-acetyl-6-O-tosyl-β-D-glucosamine



1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(Dihydrogen phosphate)



D-Glucosamine 6-(Dihydrogen phosphate)

N-Acetyl-α-D-glucosamine

A solution of D-glucosamine hydrochloride (130 g.) in water (3 l.) and methanol (300 ml.) was stirred for 90 minutes at 5° with anion exchange resin (Amberlite I.R.400, bicarbonate form) (3600 ml.) and acetic anhydride (78 ml.).[36] The mixture was filtered and the filtrate and washings were passed through a column of cation exchange resin (Amberlite I.R.100 (H), 600 ml.). Evaporation of colourless eluate and washings gave a crystalline residue which after recrystallisation from 90% aqueous ethanol-ether (115 g., 86% of theoretical) had m.p. 209-210° (dec.) $[\alpha]_D^{18}$ +53° (4 min.) ⟶ +40° (final) (c, 1·1 in H_2O). (cf. Roseman and Ludowieg,[36] yield 77%, m.p. 210° (dec.), $[\alpha]_D$ +41·0° in H_2O (final). Kuhn and Haber[37] record m.p. 202-204°, $[\alpha]_D$ +82°⟶ +40·4° in H_2O).

Found: C, 43·4; H, 6·5; N, 6·9.

Calc. for $C_8H_{15}O_6N$ C, 43·4; H, 6·8; N, 6·3%.

Tritylation and Acetylation of N-Acetyl-α-D-Glucosamine.

(a) At 40°.

N-Acetyl-α-D-glucosamine (13 g.) in dry pyridine (45 ml.) was treated with triphenylchloromethane (17·1 g.) in the presence of 'Drierite' (1 g.) at 40° for 18 hours. Acetic anhydride (24 ml.) was added and the mixture kept at 40° for a further 24 hours. The reaction mixture was filtered slowly

into vigorously stirred ice-water. After stirring for 2 hours the white granular powder which had been deposited was removed to fresh ice-water and stirred for 30 minutes. The dry white amorphous solid (30·5 g., 88% of theoretical) had $[\alpha]_D^{18}$ +46° (c, 1·3 in $CHCl_3$). The crude product was dissolved in hot chloroform and light petroleum added to turbidity. On standing crystals were deposited (5·7 g., 16·5% of theoretical) which had m.p. 245-247°, $[\alpha]_D^{19}$ -20° (c, 1·2 in $CHCl_3$). On recrystallisation from chloroform-light petroleum this product, 3,4-di-O-acetyl-N-acetyl-1,6-di-O-triphenylmethyl-D-glucosamine, had m.p. 254-256°, $[\alpha]_D^{22}$ -29° (c, 1·0 in $CHCl_3$).

Found: C, 75·9; H, 6·0; N, 2·2; Tr, 60·8.

$C_{50}H_{47}O_8N$ requires C, 76·0; H, 6·0; N, 1·8; Tr, 61·6%.

This compound was only slightly soluble in chloroform and was non-reducing to Fehling's solution.

The reducing test was carried out as follows: 1 drop of mixed Fehling's solution and 2 drops water were added to 1-2 mg. sugar derivative dissolved in 4 drops hot ethanol (freed from aldehydes by distillation over sodium). The mixture was then heated at 100° for several minutes. The following compounds were tested:

Compound	Result
1,3,4,6-Tetra-O-acetyl-N-acetyl-β-D-glucosamine	+ ve
1,2,3,4-Tetra-O-acetyl-6-O-triphenylmethyl-β-D-glucose	+ ve
1,3,4-Tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-D-glucosamine	+ ve

Compound	Result
1,3,4-Tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine	+ ve
3,4-Di-O-acetyl-N-acetyl-1,6-di-O-triphenylmethyl-D-glucosamine	- ve

After removal of the dityityl ether, the addition of light petroleum to the mother liquors gave another crystalline fraction (9·3 g., 27% of theoretical). Recrystallisation from chloroform-light petroleum gave a solvate of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-D-glucosamine, m.p. 110-113°, $[\alpha]_D^{18}$ +91° (c, 1·0 in $CHCl_3$).
Found: C, 66·6; H, 6·0; N, 2·6; Cl, 2·7%.

For complete removal of the solvent of crystallisation, it was necessary to heat the compound at 100°/15 mm. for 20 hours.
Found: loss of weight, 5·6%.

The compound, freed from solvent of crystallisation, had m.p. 154-156°, $[\alpha]_D^{18}$ +97° (c, 1·2 in $CHCl_3$).
Found: C, 66·9; H, 6·0; N, 1·8; Cl, 0.
$C_{33}H_{35}O_9N$ requires C, 67·2; H, 6·0; N, 2·4; Cl, 0%.

(b) At 100°.

Preliminary Experiment. N-Acetyl-α-D-glucosamine (10 g.) and triphenylchloromethane (13·1 g.) were heated at 100° for 30 minutes in dry pyridine (50 ml.) in the presence of

'Drierite' (1 g.). Acetic anhydride (20 ml.) was added[38] and, after standing overnight, the reaction mixture was filtered slowly with vigorous mechanical stirring into ice-water (1 l.). The amorphous solid which separated immediately was stirred for 2 hours and then for a further 30 minutes with fresh ice-water. The product (17 g.),(64% of theoretical) was filtered and dried. It had $[\alpha]_D^{16}$ +32° (c, 1·1 in $CHCl_3$).

When a hot filtered solution of the crude product in carbon tetrachloride was allowed to cool, a crystalline fraction was obtained (4 g., 15%) of theoretical) $[\alpha]_D^{18}$ +24° (c, 0·9 in $CHCl_3$). After four recrystallisations from the same solvent, this product, shown to be the carbon tetrachloride solvate of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine, lost solvent at 100-110° and had m.p. 187-189°, $[\alpha]_D^{18}$ +22° (c, 1·4 in $CHCl_3$).

Found: C, 52·3; H, 4·7; N, 2·3; Cl, 21·9.

$3C_{33}H_{35}O_9N.4CCl_4$ requires, C, 51·9; H, 4·4; N, 1·8; Cl, 23·8%.

For complete removal of the solvent of crystallisation, it was necessary to heat the compound at 100°/15 mm. for 18 hours.

Found: loss of weight, 26·2.

3C33H35O9N.4CCl4 requires loss of weight, 25·8%.

The compound, freed from solvent of crystallisation, had m.p. 189-191°, $[\alpha]_D^{18}$ +33° (c, 1·0 in $CHCl_3$).

Found: C, 66·8; H, 5·7; N, 2·0; Cl, 0.

$C_{33}H_{35}O_9N$ requires C, 67·2; H, 6·0; N, 2·4; Cl, 0%.

Large Scale Preparation. N-Acetyl-α-D-glucosamine (75 g.) in dry pyridine (500 ml.) in the presence of 'Drierite' (8 g.) was heated with triphenylchloromethane (98 g.) at 100° until the reactants dissolved (20 minutes). Acetic anhydride (150 ml.) was added and, after standing overnight, the reaction mixture was filtered and the excess of triphenylchloromethane in the filtrate decomposed with ice-water. The aqueous pyridine solution was then poured slowly with stirring into a large volume of ice-water, and the precipitated product was treated as in the preliminary experiment. After drying, the product was a white amorphous powder. (170 g., 85% of theoretical).

Solution of the crude product in the minimum of hot carbon tetrachloride, filtration, and slow cooling gave a good yield of the carbon tetrachloride solvate of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine. Recrystallisation from carbon tetrachloride gave colourless needles (68 g., 25% of theoretical) which lost solvent at 100-110° and had m.p. 187-189°, $[\alpha]_D^{18}$ +22° (c, 1·1 in $CHCl_3$).

After removal of the β anomer, the solvent was evaporated from the mother liquors and the residual syrup, dissolved in warm chloroform, was treated with an equal volume of ether. On standing, a crystalline product (11·0 g., 5·5% of theoretical) was obtained. This had m.p. 111-112° $[\alpha]_D^{20}$ +90° (c, 0·9 in $CHCl_3$) and was identical with the solvate of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-

D-glucosamine isolated in the previous experiment.(97)

The residual mother liquors were then warmed and light petroleum added to turbidity. On cooling crystalline 3,4-di-O-acetyl-N-acetyl-1,6-di-O-triphenylmethyl-D-glucosamine was deposited (4 g., 2% of theoretical). This had m.p. and mixed m.p. with the ditrityl ether previously isolated 254-256°, $[\alpha]_D^{17}$ -28° (c, 1·3 in $CHCl_3$).

Attempted Conversion of the α-Tetra-acetate to the β-Anomer.

(a) A solution of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-D-glucosamine (1·02 g., $[\alpha]_D^{18}$ +85°) in dry pyridine (5 ml.) was heated to 100°, treated with acetic anhydride (1 ml.) and allowed to cool. The filtered reaction mixture was poured into ice-water and the mixture stirred for 2 hours. The washed and dried precipitate (0·67 g.) had $[\alpha]_D^{19}$ +86° (c, 0·8 in $CHCl_3$).

(b) The above experiment was repeated starting with 0·6 g. of tetra-acetate, the temperature being maintained at 100° for 1 hour after the addition of the acetic anhydride. Again starting material was recovered (0·46 g.) $[\alpha]_D^{19}$ +86° (c, 1·0 in $CHCl_3$).

Removal of the Trityl Residues from 3,4-Di-O-acetyl-N-acetyl-1,6-di-O-triphenylmethyl-D-glucosamine.

(a) 3,4-Di-O-acetyl-N-acetyl-1,6-di-O-triphenylmethyl-D-glucosamine (1·0 g.) in glacial acetic acid (4 ml.) was cooled

to 5° and treated with a cooled saturated solution of hydrogen bromide in glacial acetic acid (0·4 ml.).[47] After shaking and cooling for 60 seconds, the reaction mixture was filtered free from precipitated triphenylbromomethane and the filtrate was poured immediately into ice-water (100 ml.). A precipitate (145 mg.) of unchanged material was deposited and removed by filtration. The aqueous filtrate was extracted with chloroform (4 x 100 ml.) and the combined chloroform extracts were washed with water (2 x 50 ml.), dried, and evaporated to a syrup (20 mg.).

(b) Hydrogen chloride was bubbled through a solution of the ditrityl ether (0·99 g.) in chloroform (20 ml.) for 15 minutes and the mixture was kept at 4° for several hours.[39] No crystalline material separated and fractionation between water and chloroform failed to yield a crystalline product.

(c) A solution of the ditrityl ether (280 mg.) in aldehyde-free ethanol (50 ml.) was shaken with hydrogen at 40° for 16 hours in the presence of platinum (from 60 mg. of Adam's platinum oxide).[39] After removal of the catalyst the solution was evaporated to dryness. The residual solid was extracted twice with light petroleum to remove triphenylmethane and twice with water. The aqueous extracts were evaporated to dryness, the residue treated with water (2 ml.), and the mixture filtered. Evaporation gave a syrup (10 mg.).

(d) The ditrityl ether (1·01 g.) was dissolved in glacial acetic acid (18 ml.) at 100°, water (12 ml.) was

added dropwise and the temperature maintained at 100° for 60 minutes.(40) On cooling crystals of triphenylcarbinol were deposited. The remaining triphenylcarbinol and unchanged starting material were precipitated by the addition of water (60 ml.). Filtration and evaporation of the filtrate gave a syrupy residue which was treated with water (2 ml.) and filtered. Evaporation of the clear filtrate gave a syrup (390 mg.). Trituration of this syrup with chloroform caused partial crystallisation. The crystals isolated (150 mg., 38% of theoretical) had m.p. 175-183°. After recrystallisation from acetone-light petroleum, the crystals gave a negative qualitative test for the trityl group(41) and were identified as 3,4-di-O-acetyl-N-acetyl-α-D-glucosamine, m.p. 186-187°, $[\alpha]_D^{18}$ +75° (c, 0·9 in $CHCl_3$), $[\alpha]_D^{18}$ +65° (5 min.)→ +35° (final) (c, 1.0 in H_2O).

Found: C, 47·2; H, 6·3; N, 4·5.

$C_{12}H_{19}O_8N$ requires C, 47.2; H, 6.3; N, 4.6%.

Removal of the Trityl Residue from 1,3,4-Tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-D-glucosamine.

(a) A solution of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-α-D-glucosamine (0·5 g.) in glacial acetic acid (2 ml.), cooled to 10°, was treated with a saturated solution of hydrogen bromide in acetic acid (0·2 ml.) and the mixture shaken for 45 seconds with cooling. The

precipitated triphenylbromomethane was filtered off and the filtrate immediately poured into ice-water (100 ml.). The slight precipitate which formed was separated and the filtrate extracted with chloroform (4 x 100 ml.). The combined chloroform extracts were washed with water (4 x 100 ml.) and dried over magnesium sulphate. Evaporation of the dried extracts gave a syrup (20 mg., 8·5% of theoretical).

(b) The trityl ether (0·4 g.), dissolved in glacial acetic acid (8 ml.), was heated to 100°. Water (5 ml.) was added dropwise and the heating continued for 60 minutes. After cooling and addition of water (25 ml.), the mixture was filtered and the residue washed with water. The combined filtrate and washings were evaporated to give a syrup (225 mg., 95% of theoretical). Extraction with water (3 ml.), filtration through filtercel-charcoal, and evaporation gave a syrup (170 mg.) which could not be crystallised.

(c) The trityl ether (0·7 g.) in aldehyde-free ethanol (55 ml.) was shaken with platinum-charcoal (containing 60 mg. Pt) in the presence of hydrogen at a pressure slightly greater than one atmosphere. After 9 hours at room temperature the catalyst was removed and the solution evaporated to dryness. The solid residue was extracted with cold light petroleum to remove triphenylmethane (100 mg., 34% of theoretical). The residue was then extracted with water (2 x 20 ml.) and the extracts were evaporated to a syrup (125 mg., 30% of theoretical) which could not be crystallised.

(d) A solution of the trityl ether (2·0 gm.) in ethyl acetate (40 ml.) was added to ethyl acetate containing platinum (50 mg.) (added as Adam's platinum oxide and reduced in situ). The mixture was shaken with hydrogen as before at room temperature for 10 hours. Removal of the catalyst followed by evaporation of the solvent gave a solid residue which was extracted with light petroleum and then with water. The aqueous extracts on evaporation gave a syrup (10 mg.).

Removal of the Trityl Residue from 1,3,4-Tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine.

In all the following experiments with this trityl ether, the starting material was weighed, and added, as its carbon tetrachloride solvate.

Preliminary Experiments. (a) 1,3,4-Tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine (20 g.) was dissolved in glacial acetic acid (36 ml.) at 100°, water (24 ml.) added dropwise, and heating at 100° continued for 60 minutes. After cooling and addition of water (120 ml.) the mixture was filtered and the filtrate evaporated to a syrup (800 mg.). The cooled solution of this syrup in ethanol yielded crystals of 3,4-di-O-acetyl-N-acetyl-α-D-glucosamine (330 mg., 43% of theoretical) m.p. 184-186°. On recrystallisation from ethanol, this product had m.p. 188-189°, mixed m.p. with 3,4-di-O-acetyl-N-acetyl-α-D-glucosamine prepared from the

1,6-di-trityl ether 186-188°, $[\alpha]_D^{19}$ +68° (5 min.)$\longrightarrow$ +32° (final) (c, 1·0 in H_2O).

Found: C, 47·1; H, 6·2; N, 4·7.

$C_{12}H_{19}O_8N$ requires C, 47·2; H, 6·8; N, 4·6%.

This material was undoubtedly 3,4-di-O-acetyl-N-acetyl-α-D-glucosamine produced by detritylation and partial deacetylation. 1,3,4,6-Tetra-O-acetyl-N-acetyl-β-D-glucosamine was subsequently shown to be hydrolysed under the above conditions, the recovery of starting material being only 33%.

(b) A solution of the trityl ether (2·0 g.) in glacial acetic acid (6 ml.) was cooled to 10° and treated with a cooled saturated solution of hydrogen bromide in glacial acetic acid (1·0 ml.). The mixture was shaken for 45 seconds with cooling and the precipitated triphenylbromomethane removed. On pouring into ice-water the filtrate gave a slight precipitate which was removed and the filtrate extracted with chloroform (2 x 100 ml.). The combined chloroform extracts were washed with water (3 x 50 ml.), dried and evaporated to a syrup (10 mg.). The aqueous filtrate after extraction with chloroform was combined with the water washings of the chloroform extract and the whole exhaustively extracted with chloroform (14 x 100 ml.). The combined extracts were dried without washing and evaporated to dryness to give a syrup (340 mg.). Solution in ethanol and addition of light petroleum gave a crop of crystals (60 mg.) m.p. 166-170° $[\alpha]_D^{18}$ +24° (c, 1·1 in H_2O).

A qualitative test for the trityl group was negative. The mother liquors were evaporated to dryness and the residue, on solution in hot benzene and cooling, yielded a further crop of crystals (50 mg.) m.p. 165-170°. The total yield (110 mg.) was 12% of theoretical.

(c) The trityl ether (5·0 g.) in glacial acetic acid (16 ml.) was cooled and treated as before with hydrogen bromide in acetic acid (1·5 ml.). After removal of the triphenylbromomethane the acetic acid solution was poured into water (100 ml.) and the resulting cloudy solution filtered and the precipitate washed. The combined filtrate and washings (200 ml.) were extracted with chloroform and the extracts dried and concentrated to a syrup. To remove residual chloroform and acetic acid, the syrup was treated with dry toluene and the solvent removed by distillation under reduced pressure. Repetition of this treatment gave a crystalline residue. The yields from the various chloroform extractions were as follows:

4 x 200 ml. $CHCl_3$	1·11 g.	(51% of theoretical)
4 x 200 ml. $CHCl_3$	0·34 g.	(16% " ")
4 x 200 ml. $CHCl_3$	0·04 g.	(2% " ")

The total yield (1·5 g.) was 69% of theoretical. After 2 recrystallisations from chloroform-ether, this compound, 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine, had m.p. 175-176°, $[\alpha]_D^{18}$ +5·5° (c, 1·6 in $CHCl_3$) and $[\alpha]_D^{18}$ +18° (c, 1·1 in H_2O).

Found: C, 48·1; H, 6·2; N, 4·0.

$C_{14}H_{21}O_9N$ requires C, 48·4; H, 6·1; N, 4·0%.

Large Scale Preparation. The large scale preparation of 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine was carried out on 5 portions (each 10 g.) of the carbon tetrachloride solvate of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine as follows:

A cooled solution of the trityl ether (10 g.) in glacial acetic acid (32 ml.) was treated with a cooled saturated solution of hydrogen bromide in glacial acetic acid (3.2 ml.). The mixture was shaken with cooling for 60 seconds and, after removal of the precipitated triphenylbromomethane, the filtrate was poured into ice-water (200 ml.). The resulting solution was filtered and the combined filtrate and washings (ca. 300 ml.) were extracted with chloroform (8 x 300 ml.). The extracts were dried without washing and evaporated to a syrup. This syrupy residue on treatment with toluene as before gave a crystalline product of sufficient purity for further use, m.p. 169-171°.

Overall yield 14·6 g. from 50 g. (67% of theoretical).

Tritylation of 1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine.

(a) A solution of 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine (50 mg.) in pyridine (1 ml.) was treated with triphenylchloromethane (40 mg.) at room temperature for 48 hours in the presence of 'Drierite' (200 mg.). The reaction

mixture was filtered and the filtrate poured into ice-water. A white powder (10 mg.) was precipitated. Solution in the minimum quantity of hot carbon tetrachloride and cooling gave the characteristic crystals of triphenylcarbinol admixed with a little of the impure 6-trityl ether. 1,2,3,4-Tetra-O-acetyl-β-D-glucose (50 mg.) under the same conditions also gave a negligible quantity of the 6-trityl derivative. The product isolated (18 mg.) consisted mainly of triphenylcarbinol.

(b) A solution of 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine (200 mg.) in pyridine (2 ml.) was treated with triphenylchloromethane (160 mg.) at 50° for 60 hours. The product isolated on pouring into ice-water was a white powder (220 mg.). This was dissolved in the minimum quantity of hot carbon tetrachloride and the solution filtered. On cooling needle shaped crystals of 1,3,4-tri-O-acetyl-N-acetyl-6-O-triphenylmethyl-β-D-glucosamine separated (150 mg., 33% of theoretical). They lost solvent at 100-110° and had m.p. 185-187°, mixed m.p. with authentic sample (after loss of solvent at 100-110°) 185-188°, $[\alpha]_D^{18}$ +24° (c, 1·2 in $CHCl_3$).

1,3,4-Tri-O-acetyl-N-acetyl-6-O-toluene-p-sulphonyl-β-D-glucosamine.

A solution of 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine (100 mg.) in pyridine (1 ml.) was treated with toluene-p-

sulphonyl chloride (110 mg.) at room temperature for 72 hours in the presence of 'Drierite' (200 mg.). The reaction mixture was filtered, the excess of acid chloride decomposed with a few drops of ice-water and the resulting solution poured into ice-water. The mixture was extracted with chloroform (3 x 150 ml.) and the extracts, after washing with 2 N. sulphuric acid (3 x 50 ml.), saturated sodium bicarbonate solution (3 x 50 ml.) and water (3 x 50 ml.), were dried and concentrated to a syrup (105 mg.). Trituration with methanol induced crystallisation. After recrystallisation from acetone-light petroleum, 1,3,4-tri-O-acetyl-N-acetyl-6-O-toluene-p-sulphonyl-β-D-glucosamine had m.p. 170-171° $[\alpha]_D^{17}$ +16° (c, 1·1 in $CHCl_3$).

Found: C, 50.7; H, 5·4; S, 6·1.

$C_{21}H_{27}O_{11}NS$ requires C, 50·3; H, 5·4; S, 6·4%.

This material (10 mg.) dissolved in dry acetone (0·1 ml.) was heated in a sealed tube with sodium iodide (10 mg.) at 100° for 2 hours. The reaction mixture deposited characteristic plate-shaped crystals of sodium toluene-p-sulphonate, indicating the presence of a primary toluene-p-sulphonyloxy group.

Tritylation of 3,4-Di-O-acetyl-N-acetyl-α-D-glucosamine.

A solution of 3,4-di-O-acetyl-N-acetyl-α-D-glucosamine (51 mg.) in pyridine (1 ml.) was treated with triphenylchloromethane (60 mg.) in the presence of 'Drierite' (100 mg.).

After standing at room temperature for 14 days, the product was isolated from the reaction mixture as a white crystalline solid (65 mg.). This on recrystallisation from carbon tetrachloride had m.p. 178-180° $[\alpha]_D^{14}$ +31° (c, 0·7 in $CHCl_3$). $[\alpha]_D^{14}$ +52° (c, 0·6 in 90% aqueous ethanol). No mutarotation in aqueous ethanol was observed.

Found: C, 66·0; H, 6·0; N, 2·5.

Calc. for $C_{31}H_{33}O_8N$ C, 68·0; H, 6·1; N, 2·6%.

Lack of material precluded identification of this product which did not appear to be either the expected derivative, 3,4-di-O-acetyl-N-acetyl-6-O-triphenylmethyl-D-glucosamine, or the 1,6-dityrityl derivative.

1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(diphenyl phosphate).

The phosphorylating agent used was diphenyl phosphorochloridate prepared by the method of Foster, Overend and Stacey.[42] Fractional distillation with a Vigreux column gave a product which had b.p. 184-185°/15 mm., n_D^{18} 1·5509. The aniline derivative was prepared and this had m.p. 128-129°. Foster, Overend and Stacey record b.p. 180-190°/15 mm., n_D^{15} 1·5505 for diphenyl phosphorochloridate and m.p. of aniline derivative 128-129°.

Preliminary Experiment. 1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine (1·0 g.) was dissolved in dry pyridine (4 ml.) in the presence of 'Drierite' (0·2 g.) and cooled to 0°.

Diphenyl phosphorochloridate (0·85 g.) was added with shaking and cooling over 20 minutes and the mixture kept at 0° for a further 15 minutes.(26) After standing overnight at 10°, the solution was filtered and cooled to 0° and the excess of acid chloride decomposed by the addition of a few drops of ice-water. After standing for 30 minutes the solution was poured slowly with stirring into ice-water. The product separated as a gel-like material which changed on stirring to a white crystalline solid (0·95 g., 57% of theoretical) which had m.p. 137-138°. After recrystallisation from aqueous acetone, the diphenyl phosphate had m.p. 144-145°, $[\alpha]_D^{18}$ +25° (c, 0·7 in $CHCl_3$).

Found: C, 53·6; H, 5·3; N, 2·7; P. 5·4.

$C_{26}H_{30}O_{12}NP$ requires C, 53·9; H, 5·2; N, 2·4; P, 5·3%.

Large Scale Preparation. 1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine (11·0 g.) was phosphorylated as in the preliminary experiment (pyridine, 33 ml., diphenyl phosphorochloridate 9·4 g.) and gave, on pouring into water, the crystalline diphenyl phosphate (12·4 g.) m.p. 137°. After recrystallisation from aqueous acetone, the product (11·8 g.) had m.p. 144-145°. The aqueous filtrate was extracted with chloroform (2 x 250 ml.) and the extracts were washed successively with 2N. hydrochloric acid (2 x 50 ml.), saturated sodium bicarbonate solution (2 x 50 ml.) and water (2 x 50 ml.), dried, and evaporated to a syrup. A cooled solution of this syrup in aqueous acetone gave a

further crop of crystals (1·1 g.) identical with the main product. The total yield (12·9 g.) was 70% of the theoretical.

1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(dihydrogen phosphate).

Preliminary Experiment. A solution of 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(diphenyl phosphate) (430 mg.) in aldehyde-free ethanol (25 ml.) was refluxed for 25 minutes with activated charcoal (0·5 g.). The charcoal was removed and the solution added to ethanol (15 ml.) containing the platinum catalyst (from 40 mg. Adam's platinum oxide reduced *in situ*). The solution was shaken in an atmosphere of hydrogen at a pressure slightly greater than one atmosphere until the uptake of hydrogen ceased (40 minutes). The uptake of hydrogen, corrected to N.T.P. (132·5 ml.) corresponded to 8 mol. (theoretical uptake 8 mol.). The catalyst was removed and the product, after evaporation of the solvent at 25°, was obtained as a semi-crystalline mass. Trituration with acetone gave a crystalline product (230 mg., 73% of the theoretical) which had m.p. 164-166° (decomp.), $[\alpha]_D^{19}$ +25° (c, 1·0 in H_2O).

Found: C, 38·9; H, 5·0; N, 3·6; P. 7·0.

$C_{14}H_{22}O_{12}NP$ requires C, 39·3; H, 5·2; N, 3·3; P, 7·3%.

Large Scale Preparation. The diphenyl ester (12·8 g.) was treated as above, in three separate portions (each 4·2 g.) with a total quantity of ethanol (100 ml.) and Adam's platinum oxide (1·3 g.). In each experiment the uptake of hydrogen reached 7·6 mol. (95% of the theoretical) and stopped. The time required for this uptake varied considerably, 18, 19 and 7 hours being required respectively for the three experiments. Removal of the solvent at low temperature gave a syrup which on treatment with ethanol gave the crystalline product (6·40 g.). The mother liquors on concentration yielded a further crop of crystals (0·69 g.). The total yield (7·09 g.) was 75% of the theoretical.

Cyclohexylamine salt. A solution of the above phosphate (50 mg.) in ethanol was treated with cyclohexylamine (1 drop) in ethanol. Addition of ether to the mixture gave a crystalline product, m.p. 183-184°. This could not, however, be recrystallised, and all subsequent attempts to prepare this derivative failed.

D-Glucosamine 6-(Dihydrogen Phosphate).

(a) 1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (450 mg.) in N hydrochloric acid (11 ml.) was heated at 100° until the rotation became constant. The final value of the rotation corresponds to $[\alpha]_D^{30}$ +53·5° (c, 2·5 in N HCl) for the dipolar ion of

Time (mins.)	α_D(1 dm.)
0	+0·71°
5	+1·74°
15	+1·34°
25	+1·33°
35	+1·33°

glucosamine 6-phosphate. Concentration of the solution to small volume and the addition of ethanol and acetone failed to induce crystallisation and attempts to prepare a crystalline cyclohexylamine salt were also unsuccessful.

(b) The hydrolysis was repeated with 1·0 g. of the phosphoric ester and N hydrochloric acid (25 ml.). After 30 minutes at 100°, the reaction solution was neutralised with barium hydroxide solution to pH 7. The neutral solution was treated with ethanol (4 volumes) and the precipitated barium salt was isolated and washed successively with 90% aqueous ethanol, ethanol, ethanol-ether (75:25), ethanol-ether (25:75), and ether. The dried precipitate (1·9 g., 206% of theoretical) was purified by solution in water (15 ml.) and reprecipitation with ethanol (60 ml.). The precipitate, washed with ethanol-ether as before, was obtained as a dry white powder (640 mg.).

Found: P, 3·5.

$C_6H_{12}O_8NPBa$ requires P, 7·87%.

This was purified further by solution in water (18 ml.), and reprecipitation with ethanol (72 ml.). After washing as before a white powder (240 mg.) was obtained.
Found: P, 6·6%.

Repetition of this procedure gave a product (80 mg., 9% of theoretical) which, after drying at 78°/15 mm., had P, 7·6%.
Found: C, 20·8; H, 4·3; N, 2·9; P, 7·6.
$C_6H_{12}O_8NPBa$ requires C, 18·3; H, 3·1; N, 3·6; P, 7·87%.

The preparation of a crystalline dibrucine salt was attempted by treatment of the barium salt (40 mg.) in water (5 ml.) with a solution of brucine sulphate (103 mg.) in water (40 ml.). The precipitated barium sulphate was removed by centrifugation and the clear solution concentrated to a glass. Trituration with ethanol gave an amorphous solid (50 mg.) which could not be crystallised.
Found: P. 3·7.
$C_6H_{14}O_8NP.2(C_{23}H_{26}O_4N_2)$ requires P, 3·0%.

(c) 1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (400 mg.) was hydrolysed with N sulphuric acid (12 ml.) at 100° for 3 hours.

Time (mins.)	α_D (1 dm.)
0	+0.58°
30	+1·10°
90	+1·18°
150	+1·21°
180	+1·21°

The final value of the rotation corresponded to $[\alpha]_D^{30}$ +60° (c, 2·0 in N H_2SO_4) for the dipolar ion of glucosamine 6-phosphate. The reaction solution was neutralised by shaking overnight with finely divided barium carbonate. After filtration and thorough washing of the barium salts with water, the combined filtrates were treated with ethanol (4 volumes) and the resulting precipitate washed and dried as in previous experiments.
Found: P, 6·3.
$C_6H_{12}O_8NPBa$ requires P, 7·9%.

The product, a white powder (290 mg.), was dissolved in 0·01N hydrochloric acid (6 ml.) and treated with ethanol (24 ml.). The resulting precipitate (150 mg., 41% of theoretical), had $[\alpha]_D^{18}$ of the dipolar ion +53° (c, 0·4 in H_2O pH 2·5). (Brown[33] records $[\alpha]_D^{24}$ +48·5° (c, 0·5 in H_2O pH 2·5) for the dipolar ion).
Found: C, 19·6; H, 4·4; N, 4·7; total P, 7·82; inorganic P, 0·05.
$C_6H_{12}O_8NPBa$ requires C, 18·3; H, 3·1; N, 3·6; total P, 7·87; inorganic P, 0%.

(d) 1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (400 mg.) was hydrolysed with N sulphuric acid (12 ml.) at 100° for 2·5 hours. The hydrolysis solution was neutralised with barium hydroxide solution (half saturated). During the neutralisation, samples (1 drop) were removed and tested for the presence

Please give to Tom & Ken to Dr Hay.

DR. G. WEILER. DR. F. B. STRAUSS.
MICROANALYTICAL LABORATORY.
164. BANBURY ROAD.
OXFORD.
TELEPHONE 57715.

Chemistry Department,
University of Edinburgh,
West Mains Road,
Edinburgh.9.

25th October, 1956

ANALYTICAL RESULTS.

5873	B.P.21	4.025mg	3.750mg CO_2	1.975mg H_2O	0.445mgRes.
5874	SL.40	3.590	10.730	1.645	
5875	SM.74	3.700	7.030	1.460	
5876	S.G.104	4.265	14.560	2.480	
5877	HH.57	4.120	7.800	1.830	
5878	58	3.805	7.205	1.635	
5879	59	3.690	7.285	1.585	
5880	60	3.430	6.715	1.745	
5881	61	2.570	5.600	1.035	
5882	S.G.105	4.235	0.150	2.795	
5875	SM.74	5.306mg	3.261mg SO_4	20.50% S	
5876	S.G.104	3.782mg	No Cl. found.		
5876	S.G.104	0.430mg	4.345mg Camphor 15.1°		240 M.Wt.
5873	B.P.21	3.860mg	0.201ml N_2	764/20°	6.10% N
5874	SL.40	3.835	0.382	764/20	11.70
5875	SM.74	3.200	0.230	764/20	8.40
		2.900	0.199	757/19	8.00
5882	S.G.105	3.690	0.1107	764/19	35.20

Substances have been dried according to instructions.

of barium ions with a solution of rhodizonic acid in water.[58] When all the sulphuric acid had been precipitated as barium sulphate, the presence of soluble barium was shown by a red-brown precipitate of barium rhodizonate. The precipitated barium sulphate was removed by centrifugation and the clear solution (pH 4) was concentrated to 10 ml. Ethanol was added to turbidity and the mixture kept at 0° for several days. Crystals were deposited (120 mg., 46% of theoretical) and these, after drying at 18°/0·1 mm. had decomp. point 170-180° $[\alpha]_D^{21}$ +54° (c, 0·5 of the hydrate in H_2O), calc. for the anhydrous compound +58°. Analysis indicated these to be the monohydrate of D-glucosamine 6-(dihydrogen phosphate).

Found: C, 25·8; H, 5·7; N, 5·2; total P, 11·3;
inorganic P, 0.

$C_6H_{14}O_8NP.H_2O$ requires C, 26·0; H, 5·8; N, 5·1; total P, 11·2;
inorganic P, 0%.

(e) 1,3,4-Tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (2·8 g.) was hydrolysed with N sulphuric acid and the hydrolysate neutralised as in preliminary experiment (d). On treatment of the neutralised solution with ethanol, the monohydrate of glucosamine 6-(dihydrogen phosphate) separated as crystals (0·81 g., 45% of theoretical).

Found: P, 11·3.

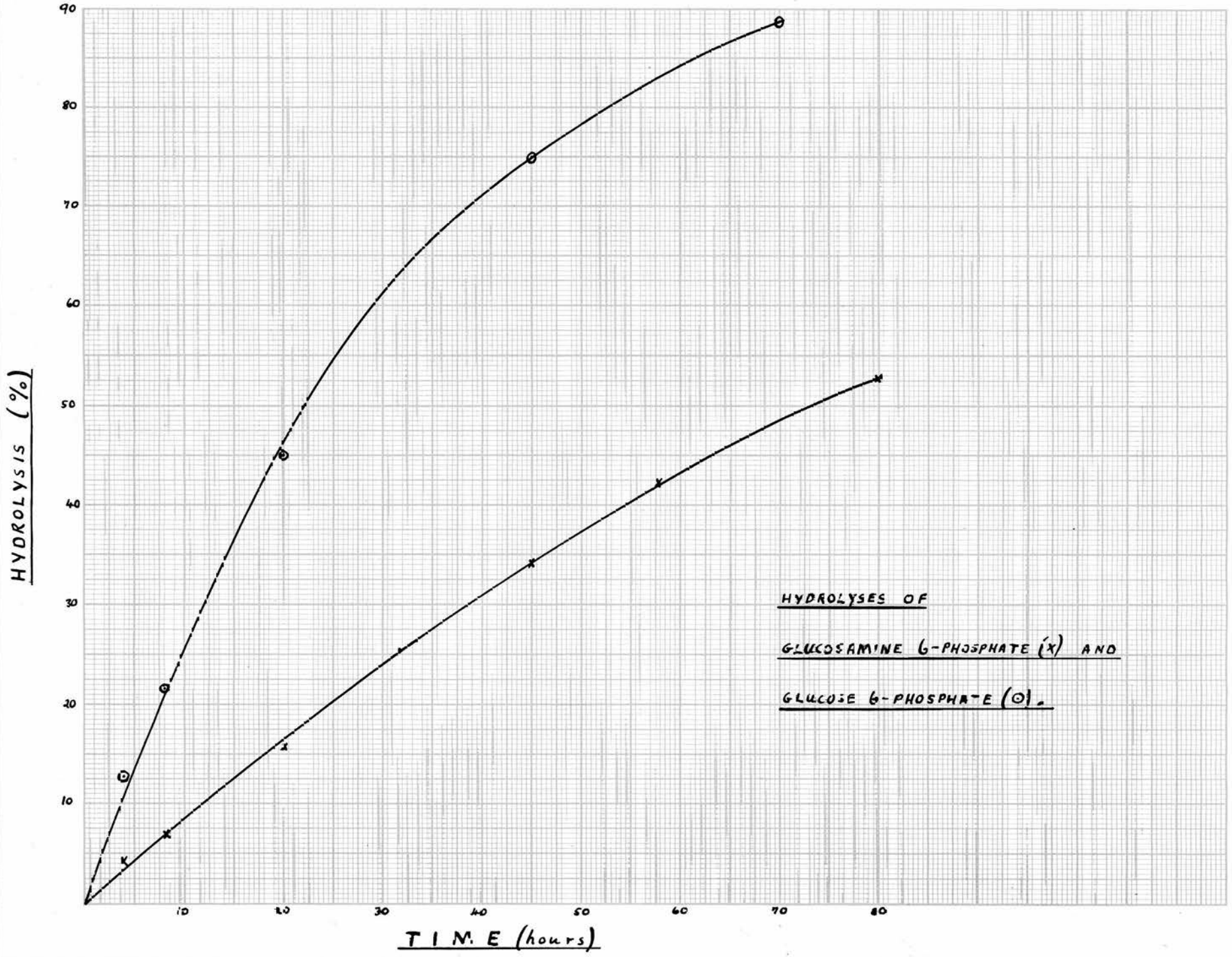
$C_6H_{14}O_8NP.H_2O$ requires P, 11·2%.

Chromatographic examination of this product (applied in dilute sulphuric acid solution) and the barium salt previously obtained (freed from barium by treatment with sulphuric acid) showed identical spots. The chromatograms were run using the ascending paper technique[83] and with ethyl acetate-pyridine-formamide (6:1:3) as solvent. The spray used was ferric chloride-sulphosalicylic acid.[74]

(f) A solution of 1,3,4-tri-O-acetyl-N-acetyl-β-D-glucosamine 6-(dihydrogen phosphate) (200 mg.) in water (3 ml.) was heated at 100° for 30 hours. The resulting brown solution, after decolourisation with charcoal, was filtered and concentrated to a glass. The glass was dissolved in water (4 ml.) and ethanol added to turbidity. After several days the precipitate which had been deposited was removed and the filtrate treated with more ethanol and seeded with the crystalline monohydrate obtained in the previous experiment. The crystalline product which separated after standing for several weeks at 0° was isolated. This product (17 mg., 12% of theoretical) had decomp. point 165-175° and analysis indicated that it was the dihydrate of glucosamine 6-(dihydrogen phosphate).
Found: C, 24·4; H, 6·0; N, 4·6; P, 10·4.
$C_6H_{14}O_8NP.2H_2O$ requires C, 24·4; H, 6·1; N, 4·7; P, 10·5%.

Hydrolysis of D-Glucosamine 6-(Dihydrogen phosphate).

The crystalline monohydrate of glucosamine 6-(dihydrogen


HYDROLYSIS (%)
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
TIME (hours)
HYDROLYSES OF
GLUCOSAMINE 6-PHOSPHATE (X) AND
GLUCOSE 6-PHOSPHATE (⊙).

phosphate) (67·9 mg.) in N hydrochloric acid (25 ml.) was heated at 100°[60] and aliquots were analysed for hydrolysed phosphorus. Robison[60] has carried out the hydrolysis of glucose 6-(dihydrogen phosphate) under the same conditions and his percentage hydrolysis figures are shown alongside for comparison.

Time (hours)	Hydrolysed phosphorus (mg/ml)	% Hydrolysis of glucosamine 6-phosphate	% Hydrolysis[60] of glucose 6-phosphate
0	nil	0	-
4	0·013	4·3	12·7
8	0·021	6·8	21·6
20	0·048	15·6	44·9
45	0·104	34·2	75·0
58	0·128	42·1	-
70	-	-	88·9
80	0·160	52·6	-

(See graphs, P. 119)

Periodate Oxidation of Glucosamine 6-Phosphate.

(A) Periodate Uptake. The oxidations were carried out under conditions similar to those used by Brown.[32] The unbuffered oxidation solutions were adjusted to pH 4·5 and the oxidation allowed to proceed at room temperature in the dark. The following is a typical oxidation. The weighed substance (100-200 μ-moles) was dissolved in water and the solution adjusted to pH 4·5 and made up to 25 ml. A sample of this (6 ml.) was taken, 3 ml. of 0·1M sodium

metaperiodate added and the mixture made up to 10 ml. At suitable intervals, 3 ml. portions were withdrawn and treated with 0·6 g. sodium bicarbonate, 0·6 g. potassium iodide, and 5 ml. 0·1N sodium arsenite. After 10 minutes the mixture was titrated with 0·05N iodine using starch indicator. In the case of a barium salt, the barium was precipitated by addition of 3 ml. 0·1N sulphuric acid and removed prior to adjusting the pH to 4·5.

Uptake of Periodate in moles/mole of sugar

	22 hours	64 hours	94 hours
Glucosamine hydrochloride	4·8	4·9	4·9
Glucosamine 6-(barium phosphate)	3·8	3·9	3·9
Glucosamine 6-(dihydrogen phosphate)	3·9	3·9	3·9

Theory: Glucosamine 5·0 moles/mole

Glucosamine 6-phosphate 4·0 moles/mole.

(B) Formaldehyde Release. Solutions of the sugar derivatives were oxidised in buffered solutions (pH 7.5) with periodate (21 hours) and the formaldehyde released estimated under exactly the conditions advocated by O'Dea and Gibbons.[59] Three glucose solutions of different concentration were used as standards and the filter used was the Ilford spectrum yellow (606).

Sugar Derivative	Moles of HCHO/ mole of sugar	Theoretical
Glucosamine hydrochloride	0.81	1.0
N-Acetyl-D-glucosamine	0.98	1.0
Glucosamine 6-(barium phosphate)	0.00	0.0
Glucosamine 6-(dihydrogen phosphate)	0.00	0.0

Attempted Estimation of the Glucosamine Content of Glucosamine 6-phosphate.

An attempt by the method of Elson and Morgan[61] as modified by Belcher, Nutten, and Sambrook,[62] was made to determine the glucosamine content of the glucosamine 6-phosphate samples prepared. Following the method prescribed exactly, using glucosamine solutions as standards and the Ilford spectrum green (604) filter, the crystalline monohydrate of glucosamine 6-(dihydrogen phosphate) was found to have a glucosamine content 59% of the theoretical and the barium salt a content 29% of the theoretical. It was noted that the colours of the solutions obtained from the phosphate derivatives were considerably more brown than those of the glucosamine standards.

Attempted Preparation of Glucosamine 6-Phosphate by Direct Phosphorylation.

Phosphorylation

Preliminary Experiment. Metaphosphoric acid, prepared by heating A. R. syrupy phosphoric acid (7 ml.) in water (1·33 ml.) until the solution became turbid,[90] was added to dry D-glucosamine hydrochloride (6 g.) and methyl cyanide (distilled over phosphoric oxide) (20 ml.) and the mixture heated under anhydrous conditions with occasional shaking at 80° for 40 minutes.[29] Removal of the methyl cyanide by decantation and treatment of the chilled residue with isopropanol (50 ml.) and ether (50 ml.) gave a solid product which after drying was a chocolate-coloured powder (6·5 g.).

Found: P, 5%.

Large Scale Preparation. Glucosamine hydrochloride (20 g.) was stirred with metaphosphoric acid (from 25 ml. phosphoric acid) and methyl cyanide (70 ml.) at 80° for 2·5 hours. The product was isolated, as in the preliminary experiment, as a dry powder (25 g.).

Found: P, 8·4%.

This product was re-phosphorylated under exactly the same conditions for 1·5 hours and isolation as before gave a dry powder (27 g.).

Found: P, 14·2%.

Attempted Selective Hydrolysis to D-glucosamine 6-(Dihydrogen Phosphate).

(a) The phosphorylated glucosamine (3·5 g., P, 14·2%) was hydrolysed for 25 minutes with N hydrochloric acid (70 ml.) at 100°. Evaporation gave a syrup which was washed with isopropanol (2 x 25 ml.) and extracted with water (150 ml.). The filtered aqueous extract was decolourised with charcoal and passed through a column (30 cm. x 5 cm.) of anion-exchange resin (Deacidite F). The column was eluted with water (1·5 l.) until the eluate was non-reducing to Fehling's solution. The eluate was treated with N hydrochloric acid (5 ml.) and concentrated to small volume. On cooling, the residual solution deposited crystals of D-glucosamine hydrochloride (0·65 g.), $[\alpha]_D^{17}$ +95° (3 minutes)⟶ +72° (final) (c, 1·1 in H_2O). (Irvine and Earl[72] record $[\alpha]_D$ +100°⟶ +72·5° in H_2O).

The column was then eluted with N hydrochloric acid until the eluate gave a negative test for the presence of phosphorus (1500 ml.). Evaporation of the eluate and trituration of the residue with isopropanol gave a yellow powder (1·7 g.).

Found: C, 22·9; H, 4·5; N, 14·4; P, 1·4.

Calc. for $C_6H_{14}O_8NP$: C, 27·8; H, 5·4; N, 5·4; P, 12·0.

Calc. for $C_6H_{14}O_8NP.HCl$: C, 24·4; H, 5·1; N, 4·7; P, 10·5%.

($C_6H_{14}O_8NP$ = Glucosamine 6-(dihydrogen phosphate)

$C_6H_{14}O_8NP.HCl$ = Glucosamine 6-(dihydrogen phosphate) hydrochloride).

Paper chromatographic examination of this product with butanol-pyridine-water (1:1:1) as solvent and aniline oxalate as spray failed to reveal the presence of any glucosamine hydrochloride.

The resin column was eluted with a further quantity of N hydrochloric acid (1 l.) and a yellow powder (1·2 g.) was isolated from this eluate.
Found: N, 16·4; P. 0·3%.

(b) The phosphorylated glucosamine (5·9 g., P, 14·2%) was hydrolysed with N hydrochloric acid (160 ml.) at 100° for 40 minutes. The hydrolysate was evaporated to dryness and the residue washed thoroughly with isopropanol in an attempt to remove free phosphoric acid and hydrochloric acid. The residue was then extracted with water (100 ml.) and the extract filtered and treated with charcoal at 100°. Residual phosphoric acid was then removed from the colourless solution by addition of barium acetate solution followed by barium hydroxide solution until no further precipitation of barium phosphate occurred. After removal of the insoluble inorganic salts, the sugar phosphate was isolated as the barium salt by the addition of a small excess of barium hydroxide followed by precipitation with ethanol (4 volumes). Washing with 90% aqueous ethanol, ethanol, ethanol-ether (75:25), ethanol-ether (25:75), and ether left a powder (220 mg.).
Found: P, 3·8.
$C_6H_{12}O_8NPBa$ requires P, 7·87%.

Extraction with water (20 ml.) and reprecipitation with ethanol (80 ml.) left, after washing with ethanol and ether as before, a dry powder (90 mg.).

Found: P, 5·9%.

The purification process was repeated and a product (45 mg.) isolated which had $[\alpha]_D^{18}$ +24° (c, 0·7 in H_2O), $[\alpha]_D^{18}$ +43° (c, 0·2 in H_2O, pH 2·5). Brown[33] records $[\alpha]_D^{24}$ +48·5° in H_2O, pH, 2·5 for the dipolar ion.

Found: C, 17·2; H, 3·6; N, 2·9; total P, 6·9, inorganic P, 0·0; Ba, 31·0.

$C_6H_{12}O_8NPBa$ requires C, 18·3; H, 3·1; N, 3·6; total P, 7·87, inorganic P, 0·0; Ba, 34·8.

$C_6H_{12}O_8NPBa.HCl$ requires C, 16·7; H, 3·0; N, 3·2; total P, 7·2, inorganic P, 0·0; Ba, 31·8%.

Chromatographic examination of this material with n-propanol-ammonia-water (6:3:1)[73] as eluant and both aniline oxalate and ferric chloride-sulphosalicylic acid[74] as sprays revealed only one spot.

Periodate Oxidation. Using the methods already described, the periodate uptake (p.120) and the formaldehyde release (p.121) of this barium salt were determined.

Results.

	22 hours	66 hours	100 hours	
Periodate uptake	2·8	3·7	3·8	(Theory 4·0)

Formaldehyde release 0·38 moles/mole (Theory 0·0).

MISCELLANEOUS EXPERIMENTS

Phenyl Thiocarbonyl Chloride.

2N Sodium hydroxide solution (25 ml.) was added, dropwise with cooling, to a mixture of phosgene (4·85 g.) and thiophenol (5·39 g.) in dry toluene (25 ml.).(75) The toluene layer was separated, dried, and the solvent removed. The residual oil was distilled, b.p. 95-105°/12 mm. and redistillation, b.p. 100°/12 mm., gave phenyl thiocarbonyl chloride (1·99 g.).

Attempted Preparation of the Phenyl Thiocarbonyl Derivative of Glucosamine.

Glucosamine (0·40 g.) in dry pyridine (30 ml.) was treated at room temperature with phenyl thiocarbonyl chloride (0·31 ml.) in the presence of 'Drierite.' After 72 hours, the filtered reaction mixture was poured into ice-water. The resulting amorphous precipitate (79 mg., 12% of theoretical) was washed with water and dried. The aqueous fraction was extracted with chloroform (3 x 100 ml.) and the extracts, after washing with 2N hydrochloric acid and water, were dried and concentrated to a syrup (30 mg., 4% of theoretical). No crystalline product could be isolated from either the amorphous solid or the syrup.

β-D-Glucosamine.

Glucosamine hydrochloride (5·0 g.) in ethanol (57 ml.) and diethylamine (3·6 ml.) was shaken for 2 days.[76] The insoluble product was separated and again shaken with ethanol (10 ml.) and diethylamine (0·8 ml.) for 1 hour. The crystalline residue was further purified by digestion with ethanol (25 ml.) at 40° overnight and, after drying, these crystals of β-D-glucosamine had m.p. 118°, $[\alpha]_D^{22}$ +25° (3 min.) ⟶ +48° (final) (c, 1·2 in H_2O). (Westphal and Holzmann[76] record m.p. 120° $[\alpha]_D$ +20° ⟶ +47·5° in H_2O).

1,3,4,6-Tetra-O-acetyl-N-acetyl-β-D-glucosamine.

β-D-Glucosamine (3·0 g.) in pyridine (31 ml.) and acetic anhydride (19 ml.) was allowed to stand at room temperature for 5 days.[76] The mother liquors were decanted and the residual crystalline product was washed with water and dried. The crystals (2·2 g., 34% of theoretical) of 1,3,4,6-tetra-O-acetyl-N-acetyl-β-D-glucosamine had m.p. 185-187°, $[\alpha]_D$ +1° (c 1·2 in $CHCl_3$) (Hudson and Dale[77] record m.p. 187-189°, $[\alpha]_D$ +1·2° in $CHCl_3$).

Hydrolysis of N-Acetyl-D-glucosamine.

Solutions (4%) of N-acetyl-D-glucosamine in various concentrations of hydrochloric acid were hydrolysed, at

different temperatures, until the rotations of the solutions reached constant values.

(A) N. HCl at 60°

Time (hours)	a_D (1 dm.)
0	1·41°
2	1·70°
4	1·87°
22	2·41°
58	2·62°
70	2·68°

(B) N. HCl at 100°

Time (hours)	a_D (1 dm.).
0	1·41°
0·5	2·30°
1	2·49°
2	2·65°
3	2·65°

(C) 3N. HCl at 60°

Time (hours)	a_D (1 dm.)
0	1·40°
1	1·69°
2	1·88°
3	2·03°
5·5	2·21°
10·5	2·50°

(D) 3N. HCl at 100°

Time (hours)	a_D (1 dm.)
0	1·40°
0·5	2·70°
1·0	2·76°
1·5	2·76°

Hydrolysis of 1,3,4,6-Tetra-O-acetyl-N-acetyl-β-D-glucosamine.

A solution (4%) of 1,3,4,6-tetra-O-acetyl-N-acetyl-β-D-glucosamine in N hydrochloric acid was heated at 100° until the rotation reached a constant value.

Time (min.)	α_D (1 dm.)
2	1.99°
13	1.21°
25	1.29°
42	1.36°
57	1.43°
70	1.43°

The hydrolysate was concentrated to small volume and treated with ethanol (3 vol.) and acetone. Crystals of D-glucosamine hydrochloride (200 mg., 84% of theoretical) separated which had $[\alpha]_D^{18}$ +86° (3 min.) $\longrightarrow$ +70° (final) (c, 1.1 in H_2O) (Irvine and Earl[72] record $[\alpha]_D$ +100° $\longrightarrow$ +72.5° in H_2O).

SUMMARY

Part I

(1) Crystalline methyl 2:3-anhydro-α- and β-D-lyxofuranosides have been prepared (the latter for the first time) by an improved method from the crystalline methyl 3:5-isopropylidene-2-toluene-p-sulphonyl-α- and β-D-xylofuranosides. Methylation of these anhydro sugars gave the corresponding 5-methyl ethers.

(2) The methyl 2:3-anhydro-D-lyxofuranosides have been treated with methanolic ammonia and crystalline N-isopropylidene and N-acetyl derivatives of the methyl 3-amino-3-deoxy-D-arabofuranosides have been isolated and characterised. Hydrolysis of the N-acetyl derivatives gave a crystalline hydrochloride of 3-amino-3-deoxy-D-arabinose.

(3) Methylation of the methyl 3-acetamido-3-deoxy-D-arabofuranosides led to the isolation of one mono- and two di-methyl new crystalline ethers.

(4) Ammonolyses, with methanolic ammonia, of the methyl 2:3-anhydro-5-methyl-D-lyxofuranosides gave crystalline N-acetyl derivatives of the methyl 3-amino-3-deoxy-5-methyl-D-arabofuranosides which were characterised.

(5) Attempts were made to separate crystalline 2-amino-2-deoxy-D-xylose derivatives from the ammonolysis products of each of the 2:3-anhydro-D-lyxose derivatives without significant success.

Part II

(6) An attempted preparation of glucosamine 6-phosphate by tritylation and acetylation of N-p-methoxybenzylidene-D-glucosamine followed by detritylation, phosphorylation, and de-acetylation was unsuccessful, due mainly to the instability of the N-p-methoxybenzylidene group.

(7) A successful synthesis was carried out by tritylation and acetylation of N-acetyl-D-glucosamine. The α and β anomers of the tetra-acetyl-6-trityl derivative were separated. Detritylation and phosphorylation of the β anomer, followed by de-acetylation led to the isolation of the crystalline monohydrate of glucosamine 6-phosphate. Each intermediate in the synthesis was obtained crystalline.

(8) The constitution of the glucosamine 6-phosphate was confirmed by the theoretical uptake of periodate and the absence of formaldehyde on oxidation with periodate under standard conditions. Estimation of the glucosamine content of glucosamine 6-phosphate by the Elson-Morgan technique was shown to be inapplicable.

(9) The preparation of glucosamine 6-phosphate by the direct phosphorylation of glucosamine hydrochloride with metaphosphoric acid was not successful, an impure product being obtained.

REFERENCES.

(1) Meyer, Adv. Protein Chem., 1945, 2, 249.
Stacey, Adv. Carbohydrate Chem., 1946, 2, 161.

(2) Kuehl, Flynn, Holly, Mozingo, and Folkers, J. Amer. Chem. Soc., 1946, 68, 536.

(3) Baker and Schaub, J. Org. Chem., 1954, 19, 646.

(4) Ledderhose, Z. physiol. Chem., 1878, 2, 213.

(5) Tiemann, Ber., 1886, 19, 49.

(6) Levene and LaForge, J. Biol. Chem., 1914, 18, 123.

(7) Levene, J. Biol. Chem., 1916, 26, 143.

(8) Haworth, Lake and Peat, J., 1939, 271.

(9) James, Smith, Stacey and Wiggins, J., 1946, 625.

(10) Foster and Stacey, Adv. Carbohydrate Chem., 1952, 7, 247.

(11) Moggridge and Neuberger, J., 1938, 745.

(12) Neuberger and Rivers, J., 1939, 122.

(13) Freudenberg, Eich, Knoevenagel and Westphal, Ber., 1940, 73B, 441.

(14) Freudenberg, Burkhart and Braun, Ber., 1926, 59, 714.

(15) Fischer and Leuchs, Ber., 1903, 36, 24.

(16) Wolfrom and Anno, J. Amer. Chem. Soc., 1953, 75, 1038.

(17) Peat, Adv. Carbohydrate Chem., 1946, 2, 37.

(18) Müller, Moricz and Verner, Ber., 1939, 72B, 745.

(19) Baker, Schaub and Williams, J. Amer. Chem. Soc., 1955, 77, 7.

(20) Newth and Homer, J., 1953, 989.
Bose, Chaudhuri, and Bhattacharyya, Chem. and Ind., 1953, 869.
Fürst and Plattner, Abs. Papers, 12th Internat. Congr., Pure and Applied Chem., N.Y., 1951, p.409.

Newth, Chem. and Ind., 1953, 1257.

Cookson, Chem. and Ind., 1954, 223.

Angyal, Chem. and Ind., 1954, 1230.

Cookson, Chem. and Ind., 1954, 1512.

(21) Kuhn and Baer, Ber., 1953, 86, 724.

(22) Wiggins, J., 1944, 522.

(23) Raymond and Levene, J. Biol. Chem., 1929, 83, 619.

(24) Brigl and Müller, Ber., 1939, 72, 2121.

(25) Reithel, J. Amer. Chem. Soc., 1945, 67, 1056.

(26) Lardy and Fischer, J. Biol. Chem., 1946, 164, 513.

(27) Posternak and Rosselet, Helv. Chim. Acta, 1953, 36, 1614.

(28) Seegmiller and Horecker, J. Biol. Chem., 1951, 192, 175.

(29) Viscontini and Olivier, Helv. Chim. Acta, 1953, 36, 466.

(30) Harpur and Quastel, Nature, 1949, 164, 693.

(31) Grant and Long, Biochem. J., 1952, 50, xx.

(32) Brown, Biochim. Biophys. Acta, 1951, 7, 487.

(33) Brown, J. Biol. Chem., 1953, 204, 877.

(34) Leloir and Cardini, Biochim. Biophys. Acta, 1953, 12, 15.

(35) Kalckar and Klenow, Ann. Rev. Biochem., 1954, 23, 574.

(36) Roseman and Ludowieg, J. Amer. Chem. Soc., 1954, 76, 301.

(37) Kuhn and Haber, Ber., 1953, 86, 722.

(38) Reynolds and Evans, Org. Synth., 1942, 22, 56.

(39) Micheel, Ber., 1932, 65, 262.

(40) Jeanloz, J. Amer. Chem. Soc., 1952, 74, 4597.

(41) Valentin, Coll. Czechoslav. Chem. Commun., 1931, 3, 499.

(42) Foster, Overend, and Stacey, J., 1951, 980.

(43) Jolles and Morgan, Biochem. J., 1940, 34, 1183.

(44) Bergmann and Zervas, Ber., 1931, 64B, 975.

(45) Jeanloz, J. Amer. Chem. Soc., 1954, 76, 555.

(46) Jeanloz, J. Amer. Chem. Soc., 1954, 76, 558.

(47) Helferich and Klein, Ann., 1926, 450, 219.

(48) Zeile and Kruckenberg, Ber., 1942, 75B, 1127.

(49) Ehrensvärd, Nature, 1947, 159, 500.

(50) White, J., 1940, 428.

(51) Wolfrom, Burke, and Waisbrot, J. Amer. Chem. Soc., 1939, 61, 1827.

(52) Edington, Hirst, and Percival, J., 1955, 2281.

(53) Cutler, Haworth, and Peat, J., 1937, 1979.

(54) Peat and Wiggins, J., 1938, 1810.

(55) Hirst and Peat, Ann. Reports, 1934, 31, 172.

(56) Helferich and Klein, Ann., 1927, 455, 173.

(57) Tipson, Adv. Carbohydrate Chem., 1953, 8, 107.

(58) Feigl, "Spot Tests" Vol. I, Elsevier, 1954, p.203.

(59) O'Dea and Gibbons, Biochem. J., 1953, 55, 580.

(60) Robison, Biochem. J., 1932, 26, 2191.

(61) Elson and Morgan, Biochem. J., 1933, 27, 1824.

(62) Belcher, Nutten, and Sambrook, Analyst, 1954, 79, 201.

(63) Tipson, Clapp, and Cretcher, J. Org. Chem., 1947, 12, 133.

(64) Levene, Raymond, and Dillon, J. Biol. Chem., 1932, 95, 699.

(65) Percival and Percival, J., 1950, 690.

(66) Percival and Zobrist, J., 1952, 4306.

(67) Percival and Zobrist, J., 1953, 564.

(68) Cole, "Practical Physiological Chemistry," Heffer, Cambridge, 9th Ed., 1933, p.355.

(69) Sugihara, Adv. Carbohydrate Chem., 1953, 8, 26.

(70) Neuberger, Biochem. J., 1938, 32, 1435.

(71) "Organic Reagents for Organic Analyses," Hopkin and Williams Ltd., 2nd Ed., 1950, p.249.

(72) Irvine and Earl, J., 1922, 121, 2370.

(73) Hanes and Isherwood, Nature, 1949, 164, 1107.

(74) Wade and Morgan, Nature, 1953, 171, 529.

(75) Rivier, Bull. Soc. chim., 1907, 1, 733.

(76) Westphal and Holzmann, Ber., 1942, 75, 1274.

(77) Hudson and Dale, J. Amer. Chem. Soc., 1916, 38, 1431.

(78) Ingold, "Structure and Mechanism in Organic Chemistry," G. Bell and Sons Ltd., London, 1953, p.342.

(79) Viebock and Brecker, Ber., 1930, 63, 3207.
Elek, Ind. Eng. Chem. Anal., 1939, 11, 174.

(80) Clark, Ind. Eng. Chem. Anal., 1936, 8, 487; 1937, 9, 539.

(81) Allen, Biochem. J., 1940, 34, 858.

(82) Editorial Report on Nomenclature, J., 1952, 5108.

(83) Horne and Pollard, J. Bact., 1948, 55, 231.

(84) Augestad and Berner, Acta Chem. Scand., 1954, 8, 251.

(85) Winstein and Henderson, "Heterocyclic Compounds," Vol. I, John Wiley and Sons Inc., New York, 1950, p.32.

(86) Stewart and VanderWerf, J. Amer. Chem. Soc., 1954, 76, 1259.
Feldstein and VanderWerf, J. Amer. Chem. Soc., 1954, 76, 1629.

(87) Rydon and Smith, Nature, 1952, 169, 922.

(88) Wolfrom, Quinn, and Christman, J. Amer. Chem. Soc., 1934, 56, 2789.

(89) Foster, Overend, Stacey, and Wiggins, J., 1949, 2542.

(90) Viscontini, Bonetti, and Karrer, Helv. Chim. Acta, 1949, 32, 1478.

The author wishes to express his thanks to Professor E. L. Hirst, F.R.S., and Dr. E. E. Percival for their advice and encouragement, and to the Department of Scientific and Industrial Research for a Maintenance Grant. Thanks are also due to Messrs. T. & H. Smith for a gift of glucosamine hydrochloride, and Messrs. J. F. Macfarlan and Co. Ltd. for the use of an autoclave.

Preprinted from the Journal of the Chemical Society, March, 1955, (5857), pages 1042—1043.

The Ammonolysis of Methyl 2 : 3-Anhydro-D-lyxofuranoside.

By J. M. Anderson and Elizabeth Percival.

[Reprint Order No. 5857.]

Baker, Schaub, Joseph, and Williams (*J. Amer. Chem. Soc.*, 1954, **76**, 4044) in the course of a total synthesis of puromycin reported the isolation of the α- and the β-form of methyl 3-acetamido-3-deoxy-D-arabofuranoside by ammonolysis, followed by *N*-acetylation, of the methyl 2 : 3-anhydro-D-lyxofuranosides. In investigations into the mode of fission of ethylene oxide rings we too carried out this series of reactions and isolated the two anomers. Baker *et al.* (*loc. cit.*) concluded that their product was the 3-amino-3-deoxy-D-arabinose derivative and not the other possible fission product, the 2-amino-2-deoxy-D-xylose derivative, inasmuch as the hydrochloride of their product had a different decomposition point and rotation from 2-amino-2-deoxy-D-xylose hydrochloride, prepared by Wolfrom and Anno (*J. Amer. Chem. Soc.*, 1953, **75**, 1038). We obtained proof that neither of the crystalline *N*-acetyl derivatives was a xylose derivative because all attempts to condense them with acetone were unsuccessful. The starting material was obtained in quantitative yield in every experiment, whereas 2-amino-2-deoxy-D-xylose would have given the 3 : 5-*iso*propylidene derivative. It is clear therefore that the main product from the fission of the oxide ring is an arabinose derivative.

Proof that no change to the pyranose form had occurred was obtained by the isolation from both anomers of the di-*O*-toluene-*p*-sulphonyl derivative followed by replacement of the primary toluene-*p*-sulphonyloxy-group by iodine. The action of sodium iodide in acetone was carried out under conditions specific for the replacement of a toluene-*p*-sulphonyloxy-group attached to a primary carbon atom (see Tipson, *Adv. Carbohydrate Chem.*, 1954, **8**, 192). Control experiments were carried out with methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside and toluene-*p*-sulphonamide. The former gave the same percentage yield of sodium toluene-*p*-sulphonate as did the above di-*O*-toluene-*p*-sulphonyl derivatives; the latter gave no sodium toluene-*p*-sulphonate, evidence that had the toluene-*p*-sulphonyl chloride condensed with the amino-group of the sugar it would not be removed under these conditions.

Experimental.—Solvents were removed under reduced pressure. Methanolic ammonia was prepared by saturating dry methanol with ammonia at 0°.

Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside. Methyl 2 : 3-anhydro-α-D-lyxofuranoside (*J.*, 1953, 564) (m. p. 80°; 0·90 g.), dissolved in dry methanolic ammonia (40 ml.), was heated in a sealed tube at 120° for 48 hr. After concentration of the cooled solution, distillation gave a viscous syrup (0·85 g.), b. p. 150—170°/0·01 mm. A solution of this in water (20 ml.) and methanol (2 ml.) was stirred at 5° with anion-exchange resin (Amberlite I.R. 400; bicarbonate form) (24 ml.) and acetic anhydride (0·6 ml.) for 90 min. (Roseman and Ludowieg, *J. Amer. Chem. Soc.*, 1954, **76**, 301). Filtration and evaporation gave a syrup (0·96 g.), which formed prisms (0·52 g.) from acetone, and on recrystallisation had m. p. 120—121°, $[\alpha]_D^{24}$ +124° (*c*, 0·7 in EtOH), +134° (*c*, 1·0 in H_2O) (Baker *et al.*, *loc. cit.*, record m. p. 115—116°, $[\alpha]_D$ +102° in H_2O) (Found : C, 47·2; H, 7·6; N, 6·5. Calc. for $C_8H_{15}O_5N$: C, 46·8; H, 7·4; N, 6·8%).

Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside. Syrupy methyl 2 : 3-anhydro-αβ-D-lyxoside (2·8 g.), in dry methanolic ammonia (75 ml.), was treated as described for the α-form. A portion (1·01 g.) of the derived syrup in dry methanol (20 ml.) was treated with acetic anhydride (2·5 ml.), and the mixture kept at 18° for 18 hr. This, after dilution with water (10 ml.), neutralisation with solid sodium hydrogen carbonate, filtration, and evaporation to dryness, gave a residue which was extracted with chloroform. Evaporation of the dried chloroform extract gave a syrup (0·76 g.) which, on solution in methanol–acetone (50%), addition of ether to incipient turbidity and recrystallisation, yielded needles (0·16 g.), m. p. 156°, $[\alpha]_D^{18}$ −165°

(*c*, 1·0 in EtOH), −120° (*c*, 1·2 in H_2O) (Baker *et al.*, record m. p. 155°, $[\alpha]_D$ −119° in H_2O) (Found: C, 46·2; H, 7·3; N, 6·9%).

Attempted isolation of an iso*propylidene derivative.* Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (50 mg.) was shaken with dry acetone (10 ml.), anhydrous copper sulphate (1 g.), and 2 drops of acetaldehyde for 14 days. This gave a syrup (45 mg.) which crystallised completely on trituration with acetone; the solid had m. p. and mixed m. p. with starting material 118—120°, $[\alpha]_D^{15}$ +124° (*c*, 0·7 in EtOH). Methyl 3-acetamido-3-deoxy-β-D-arabofuranoside (50 mg.) was treated with dry acetone and anhydrous copper sulphate under the same conditions. The product (46 mg.) had m. p. and mixed m. p. with starting material 156°, $[\alpha]_D^{18}$ −160° (*c*, 0·5 in EtOH) (Found: C, 46·9; H, 7·35. Calc. for $C_8H_{15}O_5N$: C, 46·8; H, 7·4; for $C_{11}H_{19}O_5N$: C, 53·8; H, 7·8%).

Methyl 3-*acetamido*-3-*deoxy*-2 : 5-*di*-O-*toluene*-p-*sulphonyl*-α-D-*arabofuranoside.* Methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (10 mg.) in dry pyridine (1 ml.) was treated with toluene-*p*-sulphonyl chloride (40 mg.) in the presence of "Drierite" for 60 hr. at room temperature. The toluene-*p*-sulphonyl derivative (11·7 μmoles) obtained after appropriate treatment, was dissolved in dry acetone and heated with sodium iodide in a sealed tube for 2 hr. at 100°. Characteristic plate-shaped crystals of sodium toluene-*p*-sulphonate (identified as the *S*-benzylthiouronium salt, m. p. 179—180°) were deposited in a yield (10·3 μmoles) comparable with that in a similar experiment carried out on methyl 2 : 3 : 4-tri-*O*-benzoyl-6-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside (9·3 μmoles from/11·4 μmoles). Toluene-*p*-sulphonamide failed to yield sodium toluene-*p*-sulphonate under similar treatment. Identical experiments were carried out with the β-anomer (10 mg.) and the derived 2 : 5-di-*O*-toluene-*p*-sulphonyl derivative (19·5 μmoles) on treatment with sodium iodide in acetone gave sodium toluene-*p*-sulphonate (16·5 μmoles). (Corrections for solubility of sodium toluene-*p*-sulphonate in acetone were made in all of these experiments.)

3-*Amino*-3-*deoxy*-D-*arabinose hydrochloride.* After methyl 3-acetamido-3-deoxy-α-D-arabofuranoside (91 mg.) had been heated at 100° with hydrochloric acid (10 ml.; 3N) for 60 min. and the solution had been concentrated to dryness, a syrup was obtained which crystallised from methanol; the solid had m. p. 161° (decomp.), $[\alpha]_D^{18}$ −109° (*c*, 0·3 in H_2O) {Baker *et al.*, *loc. cit.*, record m. p. 159° (decomp.), $[\alpha]_D$ −112° in H_2O}.

The authors are grateful to Professor E. L. Hirst, F.R.S., for advice and encouragement, and one of them (J. M. A.) is indebted to the Department of Scientific and Industrial Research for a maintenance grant.

THE UNIVERSITY, EDINBURGH. [*Received, November 8th*, 1954.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY AND COMPANY, LTD., BUNGAY, SUFFOLK.

Preprinted from the Journal of the Chemical Society, April 1952, (224), pages 1243—1246.

224. *The Synthesis of Laminaribiose (3-β-D-Glucosyl D-Glucose) and Proof of its Identity with Laminaribiose isolated from Laminarin.*

By P. Bächli and (the late) E. G. V. Percival.

Laminaribiose has been synthesised by the interaction of 1 : 2-5 : 6-di*iso*propylidene glucose and tetra-acetyl glucosyl bromide, proof of its formulation as 3-β-D-glucosyl D-glucose being thus obtained. The synthetic material is identical with laminaribiose prepared from laminarin. Various derivatives of the sugar are described including the α- and the β-octa-acetate, hepta-acetyl laminaribiosyl bromide, hepta-acetyl methyl-β-laminaribioside, and methyl-β-laminaribioside.

Laminaribiose was first studied by Barry (*Sci. Proc. Roy. Dublin Soc.*, 1941, **22**, 423), who isolated it as the osazone from the mixture of sugars produced by the action of the juice of *Helix Pomatia* on the polysaccharide laminarin. The free sugar, m. p. 161—162°, was obtained by the partial hydrolysis of laminarin with mineral acid. After removal of the glucose by fermentation and of the oligosaccharides by precipitation with alcohol, the disaccharide was obtained as an amorphous powder which crystallised when its aqueous solution was slowly evaporated. Earlier work by the same author (*ibid.*, 1939, **22**, 59) had shown that laminarin was composed of glucose residues mutually linked through the 1 : 3-positions by β-linkages. Since the disaccharide was hydrolysed by emulsin giving rise only to glucose, Barry formulated it as 3-β-D-glucosyl D-glucose. Laminaribiose was further studied by Connell, Hirst, and Percival (*J.*, 1950, 3494), and its identity has now been confirmed by synthesis as the result of the condensation of 2 : 3 : 4 : 6-tetra-acetyl glucosyl bromide with 1 : 2-5 : 6-di*iso*propylidene glucose.

Gakhokidze (*J. Gen. Chem. U.S.S.R.*, 1946, **16**, 1923) had previously claimed the synthesis of the α-linked analogue of laminaribiose by the condensation of 2 : 3 : 4 : 6-tetra-acetyl glucose with 4 : 6-benzylidene 1 : 2-*iso*propylidene glucose followed by alkaline and acid hydrolysis. The formulation of this as 3-α-D-glucosyl D-glucose was established by the same author in a later paper (*ibid.*, 1949, **19**, 2100). Preliminary experiments revealed that reactions of this type are by no means easy to carry out and the presence of an effective internal desiccant has been found to be essential. Helferich, Bohn, and Winkler (*Ber.*, 1930, **63**, 989) used calcium chloride in their synthesis of gentiobiose by the interaction of tetra-acetyl glucosyl bromide and 1 : 2 : 3 : 4-tetra-acetyl glucose, and Reynolds and Evans (*J. Amer. Chem. Soc.*, 1938, **60**, 2559) found the proprietary material "Drierite" enabled gentiobiose octa-acetate to be obtained in 75—80% yield. Haskin, Hann, and Hudson (*ibid.*, 1941, **63**, 1724) have also used "Drierite" in similar reactions.

In the present work 3-β-D-glucosyl D-glucose was synthesised by the condensation of tetra-acetyl glucosyl bromide with 1 : 2-5 : 6-di*iso*propylidene glucose in benzene solution in the presence of silver carbonate, iodine, and "Drierite." Deacetylation, followed by removal of the *iso*propylidene group, and separation of the products on a cellulose column gave in 9·5% yield the crystalline α-form, laminaribiose, m. p. 198—201°, $[\alpha]_D^{16}$ 25° ⟶ +18·6° in water (compare Connell, Hirst, and Percival, *loc. cit.*).

For comparison with the synthetic material, laminaribiose was prepared from laminarin. After partial hydrolysis of the polysaccharide, laminaribiose was separated from the other products on a charcoal–"Filter Cel" column of the type described by Whistler and Durso (*J. Amer. Chem. Soc.*, 1950, **72**, 677). The crystalline product had m. p. 199—202°, and gave no depression of m. p. on admixture with the synthetic material. The two samples gave identical osazones and octa-acetyl derivatives, and their *X*-ray powder photographs were indistinguishable. The formulation of natural laminaribiose as 3-β-D-glucosyl D-glucose is, therefore, definitely established. In the course of these experiments

crystalline hepta-acetyl methyl-β-laminaribioside and methyl-β-laminaribioside were prepared.

After this work had been completed a synthesis of 3-β-glucosyl glucose was reported by Freudenberg and Oertzen (*Annalen*, 1951, **574**, 37), who used the same starting materials for the synthesis and obtained the crystalline β-form, m. p. 188—192°, $[\alpha]_D$ +7° ⟶ 20·8° in water, of laminaribiose, but were not in a position to compare directly the natural and the synthetic disaccharide. There is, moreover, little overlap between their work and ours since their experimental methods and the derivatives they describe are not the same as those recorded in the present paper.

Experimental

1 : 2-5 : 6-*Di*iso*propylidene* 3-(β-*Tetra-acetyl* D-*Glucosyl*) D-*Glucose*.—Pure 1 : 2-5 : 6-di*iso*-propylidene glucose (10 g.), silver carbonate (15 g.), and " Drierite " (30 g.; dehydrated at 240° during 2 hours) were shaken in dry benzene (80 c.c.) for 12 hours. Iodine (3 g.) was added followed by an equimolecular proportion of 2 : 3 : 4 : 6-tetra-acetyl glucosyl bromide (15·7 g.) in dry benzene (80 c.c.), added slowly during 1 hour with constant shaking in the dark. The shaking (with occasional release of carbon dioxide from the flask) was continued until a test sample gave no precipitate with alcoholic silver nitrate (70 hours). After filtration through " Filter Cel " the unchanged di*iso*propylidene glucose (2·8 g.) was removed by repeated extraction with water (4 × 100 c.c.). The aqueous extracts showed only di*iso*propylidene glucose when examined on a paper chromatogram. The solution was dried (Na_2SO_4), treated with a little charcoal, and filtered. Removal of the solvent gave a colourless glass (*A*) (16·8 g.).

3-β-D-*Glucosyl* D-*Glucose*.—The acetyl groups were removed by shaking a solution of (*A*) in methanol (80 c.c.) for 6 hours with 0·1N-sodium methoxide (10 c.c.) at room temperature, followed by neutralisation with aqueous oxalic acid. Removal of most of the methanol at 40°/15 mm. was followed by the removal of the *iso*propylidene groups by treatment of the residue with oxalic acid (0·01N; 150 c.c.) at 100°. After neutralisation with barium carbonate and filtration, the remaining ions were removed by Amberlite 1R-100H and 1R-4B ion-exchange resins. Evaporation of the water left a colourless syrup (*B*) (10·3 g.) (Found: Ac, nil). Examination on the paper chromatogram with pyridine showed the presence of much glucose (R_G 1·0) and of substances with R_G values 3·1, 2·1, 0·75 (laminaribiose), 0·4, and 0·25.

A column of powdered cellulose (50 × 2·8 cm.) was prepared, washed, and tested as described by Hough, Jones, and Wadman (*J.*, 1949, 2511). The syrup (*B*) (10·2 g.) was dissolved in butanol–pyridine–water (2 : 1 : 1). The elution was started with butanol (300 c.c.) followed by butanol–water–pyridine (6 : 1 : 1). The disaccharide appeared after elution of the column by butanol (300 c.c.) and butanol–water–pyridine (6 : 1 : 1) (2130 c.c.), and was present in the next 600 c.c. Removal of the solvent gave a white glass (1·242 g.; equivalent to a yield of 9·5% of theory); this crystallised slowly when a concentrated solution in ethanol was kept at 0°, and formed needles (1·17 g.), m. p. 196—199°. Further recrystallisation gave material, m. p. 204—206°, $[\alpha]_D^{16}$ +24·9° (20 minutes), +18·6° (9 hours, constant) in water (*c*, 2·5). Estimation by hypoiodite oxidation according to the method described by Hirst, Hough, and Jones (*J.*, 1949, 928) indicated that the material was 99·5—100% pure (Found: C, 42·0; H, 6·6. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·5%).

3-β-*Glucosyl Glucosazone*.—The disaccharide (0·075 g.) on suitable treatment gave laminaribiosazone, which recrystallised from hot water containing some pyridine as long needles, m. p. 199—201°, $[\alpha]_D^{15}$ −76·0° in ethanol (*c*, 0·5) {Connell, Hirst, and Percival (*loc. cit.*) recorded $[\alpha]_D$ −71·5° for natural laminaribiosazone; Barry and Dillon (*Proc. Roy. Irish Acad.*, 1941, **22**, 423) gave −79·6°, and Freudenberg and Oertzen, −71°} (Found: C, 54·2; H, 6·4; N, 10·4. Calc. for $C_{24}H_{32}O_9N_4$: C, 55·4; H, 6·2; N, 10·7%). The osazone acetate was prepared by Muir and Percival's method (*J.*, 1940, 1480); it formed a yellow amorphous solid.

Laminarin (20 g.) was hydrolysed by heating it with aqueous oxalic acid (750 c.c.; 0·1N) at 100° for 7 hours. After neutralisation with calcium carbonate and filtration, the solution was evaporated at 40°/15 mm. to 100 c.c. Examination of the product on a paper chromatogram [solvent: benzene–*n*-butanol–pyridine–water (1 : 5 : 3 : 3)] indicated the presence of glucose (R_G 1·0), laminaribiose (R_G 0·75), and four unknown substances having R_G 0·45 and less. The mixture was separated on a column (25 × 4 cm.) of equal parts of charcoal and " Filter Cel " (Whistler and Durso, *loc. cit.*). The charcoal and " Filter Cel " had been thoroughly mixed, and a suspension in water was poured in small quantities into the column, the lower end of which was closed with a tightly pressed layer of cotton wool. The water was allowed to drain through

between the additions. The column was washed with water (1500 c.c.) before use. Water was used as the eluant and after the removal of glucose and a portion of the unknown substance having R_G 0·45 (750 c.c.), a mixture of laminaribiose (1·03 g.) and the rest of the unknown of R_G 4·5 (200 c.c.) was obtained. Laminaribiose alone was present in the next 800 c.c. and its complete removal (2·62 g.) was effected by changing the eluant to water–ethanol (10 : 0·7) (1200 c.c.). The impure fraction of laminaribiose was passed through a smaller column (12 × 4 cm.). The first fraction contained the unknown sugar, R_G 0·45, and the laminaribiose (0·37 g.) was obtained after addition of 7% of alcohol to the eluant. The total yield of laminaribiose was 2·99 g. After crystallisation it had m. p. 204—206° not depressed on admixture with synthetic laminaribiose; $[\alpha]_D^{18}$ +26·5° (15 minutes), +19·5° (40 hours, constant) in water (*c*, 2·8). Through the kindness of Dr. C. A. Beevers, *X*-ray powder photographs of the natural and the synthetic product were obtained, a copper target and 1 hour's exposure (25 mA; 50 kv) being used; the two substances gave identical photographs (Found : C, 42·1; H, 6·8. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·5%).

Octa-acetyl β-Laminaribiose.—Laminaribiose (0·53 g.) (from laminarin) and anhydrous powdered sodium acetate (0·26 g.) were heated with acetic anhydride (3 c.c.) at 100°. After 20 minutes the powder had dissolved, and heating was continued for a further 20 minutes. The addition of water (15 c.c.) caused the precipitation of an oil which was separated from the supernatant liquor (*C*). This oil solidified very slowly when kept under cold water. The solid (0·31 g.; m. p. 135°) was crystallised by the addition of methanol to its solution in chloroform, followed by concentration. Crystallisation was initiated by the addition of ether, followed later by light petroleum (b. p. 40—60°). After five recrystallisations *octa-acetyl β-laminaribiose* was obtained as prismatic needles, m. p. 160—161°, $[\alpha]_D^{18}$ −28·8° in chloroform (*c*, 2·5) (Found : C, 49·7; H, 5·7; Ac, 50·7. $C_{28}H_{38}O_{19}$ requires C, 49·6; H, 5·6; Ac, 50·7%).

Synthetic disaccharide (0·22 g.) was treated as above and after three recrystallisations from methanol–ether gave octa-acetyl β-laminaribiose (0·08 g.), m. p. 156—158° not depressed on admixture with the material just described; $[\alpha]_D^{16}$ −25·3° in chloroform (*c*, 2·2).

Octa-acetyl α-Laminaribiose.—(a) *Laminaribiose from laminarin.* The liquor (*C*) (see previous paragraph) was neutralised (sodium hydrogen carbonate) and extracted with chloroform. On evaporation of the chloroform, crude octa-acetyl laminaribiose was obtained as a yellow oil (0·6 g.). This material was isomerised by heating it with acetic anhydride (3 c.c.) and fused zinc chloride (0·04 g.) (cf. Hudson and Johnson, *J. Amer. Chem. Soc.*, 1915, **37**, 1270). After 45 minutes the mixture was cooled and water (20 c.c.) added. On the repeated addition of water and decantation, the precipitated oil solidified. After four recrystallisations of the brown solid (0·38 g.) from chloroform–ethanol *octa-acetyl α-laminaribiose* was obtained as prismatic needles containing one molecule of ethanol of crystallisation; it had m. p. 77—78°; $[\alpha]_D^{17}$ +20° in chloroform (*c*, 3·6), calculated for the ethanol-free compound (Found : Ac, 47·5, 47·8. $C_{28}H_{38}O_{19},C_2H_6O$ requires Ac, 47·5. $C_{28}H_{38}O_{19}$ requires Ac, 50·7%). The rotation figures for the α- and the β-octa-acetate indicate that the separation of the two isomers is not quite complete (compare Hudson and Johnson, *ibid.*, 1917, **39**, 1272).

(b) *Synthetic laminaribiose.* The sugar (0·075 g.) was acetylated by the procedure described by Kruger and Roman (*Ber.*, 1936, **69**, 1832) and Nicolas and Smith (*Nature*, 1948, **161**, 349) (yield of crude octa-acetate, 51%). Recrystallisation from ethanol gave a product having m. p. 77—79°, not depressed on admixture with the octa-acetate from natural laminaribiose described above, $[\alpha]_D^{16}$ +21° in chloroform (*c*, 1·8) (Found : C, 50·0; H, 6·3; Ac, 47·9. Calc. for $C_{28}H_{38}O_{19},C_2H_6O$: C, 49·7; H, 6·1; Ac, 47·5%). Attempts to remove the ethanol from the crystals only led to its replacement by other solvents; for instance recrystallisation from methanol gave a *product* having $[\alpha]_D^{18}$ +21·5° in chloroform (*c*, 2·56), m. p. indefinite, containing one molecular proportion of methanol of crystallisation (Found : C 49·0; H, 6·2; Ac, 48·9. $C_{28}H_{38}O_{19},CH_4O$ requires C, 49·0; H, 6·0; Ac, 48·5%). Similar results were obtained with benzene.

Hepta-acetyl Laminaribiosyl Bromide.—Laminaribiose octa-acetate (0·6 g.; a mixture of the α- and the β-form) was added with shaking to glacial acetic acid (2 c.c.) saturated with hydrogen bromide at 0° (Latham, May, and Mosettig, *J. Org. Chem.*, 1950, **15**, 884). After 5 minutes, cooling was discontinued but shaking was continued until all the octa-acetate had dissolved (10 minutes). The viscous oil which separated was kept at 15° for 3 hours. Addition of dry toluene (20 c.c.) followed by its removal at 40—50°/12 mm. gave after two such operations a pale yellow solid (0·48 g., 83%). Crystallisation of this from chloroform–ethanol gave white needles (0·44 g.), m. p. 165—167°. After four recrystallisations from chloroform–ether–light petroleum *hepta-acetyl laminaribiosyl bromide* was obtained having m. p. 180·5—181·5°

$[\alpha]_D^{18}$ +85·0° in chloroform (*c*, 3·0) (Found : C, 45·15; H, 4·9; Br, 10·8. $C_{26}H_{35}O_{17}Br$ requires, C, 44·6; H, 5·0; Br, 11·4%).

Hepta-acetyl Methyl-β-laminaribioside.—Hepta-acetyl laminaribiosyl bromide (0·32 g.), dry methanol (10 c.c.), and dry benzene (10 c.c.) [dried by shaking it with " Drierite " (3 g.) for 2 hours] were shaken in the dark with dry silver carbonate (0·5 g.) and iodine (0·1 g.) until a test sample gave no precipitate with silver nitrate (20 hours) (compare Reynolds and Evans, *J. Amer. Chem. Soc.*, 1940, **62**, 66). Chloroform (50 c.c.) was added, and after filtration aided by " Filter Cel," followed by evaporation of the solvent, a red syrup was obtained which crystallised on the addition of ethanol (yield 0·255 g., 86%). Hepta-acetyl methyl-β-laminaribioside after three recrystallisations from ethanol had m. p. 164—165°, but on further heating it resolidified and melted again at 179—180°. In another experiment the substance was obtained in the first instance as needles, m. p. 183°; nevertheless on recrystallisation it showed the double melting point, 164—165° and 179—180°, and had $[\alpha]_D^{16}$ −45° in chloroform (*c*, 1·7) (Found : C, 50·0; H, 6·0; OMe, 4·3; Ac, 48·0. $C_{27}H_{38}O_{18}$ requires C, 49·8; H, 5·9; OMe, 4·8; Ac, 47·7%).

Methyl-β-laminaribioside.—Hepta-acetyl methyl-β-laminaribioside (0·408 g.) in dry methanol (10 c.c.) was boiled with sodium methoxide (1 c.c.; 0·1N) for 1 hour (Zemplen, *Ber.*, 1936, **69**, 1827). Filtration through charcoal and removal of the solvent gave a colourless syrup, which crystallised after 4 days under alcohol. Three recrystallisations from ethanol–ether gave *methyl-β-laminaribioside* as fine needles, m. p. 165—166°, $[\alpha]_D^{19}$ −28° in water (*c*, 2·5) (Found : C, 41·9; H, 7·0; OMe, 7·8. $C_{13}H_{24}O_{11},H_2O$ requires C, 41·7; H, 6·95; OMe, 8·3%).

The authors thank the Schweizerischen Schulrat, Eidgenössische Technische Hochschule, Zürich, and the University of Edinburgh for the award of an Exchange Studentship to one of them (P. B.), and Professor E. L. Hirst, F.R.S., for his interest in this work.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, December 7th*, 1951.]

PRINTED IN GREAT BRITAIN BY RICHARD CLAY AND COMPANY, LTD.,
BUNGAY, SUFFOLK.