Synthesis and Properties of 2,3-Anhydro-D-mannose and 3,4-Anhydro-D-altrose

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Treatment of benzyl 3,4,6-tri-*O*-acetyl-2-*O*-*p*-tolylsulphonyl-β-D-glucopyranoside (5) with sodium methoxide in methanol gave benzyl 2,3-anhydro-β-D-mannopyranoside (7) (65%) and benzyl 3,4-anhydro-β-D-altropyranoside (8) (29%). Hydrogenolysis of the glycoside (7) in tetrahydrofuran over palladium–charcoal yielded crystalline 2,3-anhydro-β-D-mannopyranose (9) which, in aqueous solution, rapidly underwent mutarotation to give an equilibrium mixture containing the α-pyranose (10) (23%), the β-pyranose (9) (7%), the α-furanose (12) (65%), and the β-furanose (11) (5%), whose composition was determined by g.l.c., g.l.c.–mass spectrometry, and n.m.r. spectroscopy; the assignment of anomeric configuration to the furanose isomers rests on conformational arguments. A similar mixture was obtained by hydrogenolysis of benzyl 2,3-anhydro-α-D-mannopyranoside (13). Hydrogenolysis of the glycoside (8) in methanol gave an equilibrium mixture of the α- and β-forms [(14) and (15)] of 3,4-anhydro-D-altropyranose (32·5 and 67·5%, respectively).

THE hexokinases are key enzymes in the metabolism of hexoses in all mammalian cells.[1] In some tumour cells there is evidence that the initial phosphorylation of hexose, catalysed by hexokinase, may be a rate-controlling step in hexose utilisation.[1-4] Because of the broad specificity of hexokinases it might be possible to design a hexose-like inhibitor effective in controlling glycolysis rates in tumour cells.

There have been several examples of the irreversible inhibition of carbohydrate-metabolising enzymes by agents directed to the active site of the enzyme by virtue of a structural similarity to the substrate.[5] Almond β-D-glucopyranosidase is irreversibly inhibited by 1D-1,2-anhydro-*myo*-inositol [6-8] and the β-D-xylopyranosidase of *Helix pomatia* is inactivated by 1L-1,2-anhydro-*myo*-inositol.[8] Triose phosphate isomerase is irreversibly inhibited by D-(and L-)glycidol phosphate,[9,10] and phosphoglucose isomerase by certain 1,2-anhydrohexitol 6-phosphates.[11] In these examples the inhibitor resembles an intermediate in the normal enzymic reaction. Another group of inhibitors functions by means of a reactive side-chain. 2,3-Epoxypropyl β-D-glycosides of 2-acetamido-2-deoxy-D-glucose and its oligomers are inhibitors of hen's egg-white lysozyme,[12-14] and 2,3-epoxypropyl β-D-glucopyranoside [15,16] irreversibly inhibits yeast hexokinase.[15] Very recently [17] similar epoxide

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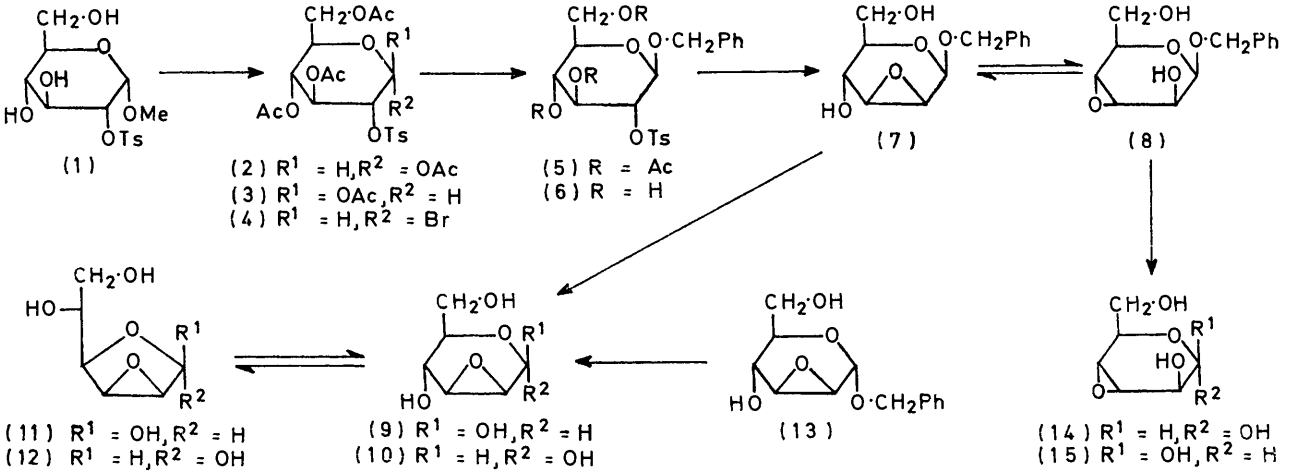
derivatives of cellobiose and cellotriose have been shown to inhibit cellulases. In all these cases nucleophilic opening of the epoxide ring occurs while the inhibitor is attached to the active site of the enzyme, resulting in covalent binding to the site.

As part of a programme concerned with the preparation of 2,3- and 3,4-anhydrohexoses as potential inhibitors of hexokinase from different tissues, we have prepared 2,3-anhydro-D-mannose and 3,4-anhydro-D-altrose.

Sugar epoxides in which the reducing group is present have not been characterised hitherto. Dekker and Hashizume attempted the preparation of 5,6-anhydro-D-glucose by cautious acidic hydrolysis of its 1,2-*O*-isopropylidene derivative,[18] but the acidic conditions caused hydrolysis and intramolecular opening of the oxiran ring to yield D-glucose and 2,5-anhydro-L-idose. These types of reaction were also observed during a more extensive study by Buchanan and Conn,[19,20] who were nevertheless able to detect a reducing sugar intermediate, presumably 2,3-anhydro-D-mannose, during the acidic hydrolysis of methyl 2,3-anhydro-α-D-mannopyranoside.[20] 2,3-Anhydroribose and 2,3-anhydrolyxose have also been partially characterised by chromatography.[21]

In order to avoid acidic conditions during the final stage of the synthesis we have used benzyl glycosides, in the expectation that hydrogenolysis of the benzyl group [22,23] would occur more readily than reduction of the oxiran ring. The general route is shown in Scheme 1.

The toluene-*p*-sulphonate (1) [24,25] was prepared from methyl α-D-glucopyranoside by minor modifications of standard methods and converted into a mixture of acetates (2) [26,27] and (3) [26-28] by acetolysis. The reaction required more vigorous conditions than usual, owing, no doubt, to the presence of the electron-withdrawing sulphonate group on O-2.[23,24,29] As expected, the α-anomer (2) preponderated. Both isomers, on treatment with hydrogen bromide in acetic acid, yielded the bromide (4),[26-28] which was converted into the β-glucoside (5) [30] by reaction with benzyl alcohol and lead carbonate. The formation of the anhydro-derivatives (7) and (8) by treatment of the toluene-*p*-sulphonate (5) with sodium methoxide (5 equiv.) in methanol could be monitored conveniently by t.l.c. The initial product was presumably the triol (6), which reacted more slowly to give the anhydromannoside (7). Epoxide migration then resulted in formation of the anhydroaltroside (8). Complete disappearance of the triol (6) was not achieved until an essentially equilibrium mixture of the two epoxides had been formed. The two epoxides (7) (65%) and (8) (29%) were isolated by crystallisation followed by chromatography. The presence of an appreciable quantity of each isomer was to be expected from the early work of Lake and Peat [31] and from recent studies on methyl 6-deoxy-anhydro-β-hexopyranosides by Drs. S. A. S. Al-Janabi and A. R. Edgar in this laboratory.[32] It is interesting that in the α-series the 2,3-anhydromannoside is strongly preferred.[33] No attempt was made to measure equilibrium constants in the present work.



SCHEME 1

Hydrogenolysis of benzyl 2,3-anhydro-β-D-mannopyranoside (7) in methanol over palladium–charcoal gave

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a product that was homogeneous by t.l.c. and by paper chromatography. Difficulty was experienced in isolating the crystalline product, but when tetrahydrofuran was used as solvent for the hydrogenation, in order to decrease the rate of mutarotation,[22] 2,3-anhydro-β-D-mannopyranose (9) could be isolated in 50% yield. The crystalline compound and the residual syrup after mutarotation had identical properties on paper chromatography and were indistinguishable from one of the products of acidic hydrolysis of methyl 2,3-anhydro-α-D-mannopyranoside.[20] The structure (9) was assigned to the crystalline compound on the basis of the result of direct hydrogenolytic cleavage of the benzyl glycoside (7).[22] Further evidence derived from various physical methods applied to the compound itself and to the products of mutarotation is consistent with this conclusion.

The equilibrium mixture was analysed by g.l.c. after conversion of the components into their trimethylsilyl (Tms) ethers.[34] Such a method has been used [35-37] to study similar equilibria and depends on the rapidity of the trimethylsilylation reaction. An aqueous solution, shown by polarimetry to have reached equilibrium, was evaporated to dryness and converted into Tms ethers. Four peaks appeared on g.l.c., designated A—D in order of elution from the column, in the ratio 65 : 23 : 5 : 7. A very similar chromatogram was obtained for the products of mutarotation in pyridine, the ratios being 58 : 27 : 3 : 12. The retention times, relative to the Tms ether of α-D-glucose, were 0·94, 1·33, 1·48, and 2·03. When the crystalline 2,3-anhydromannose was subjected to trimethylsilylation and g.l.c. the product corresponded in retention time to component D, one of the minor products of equilibration.

The compositions of equilibrating solutions of crystalline 2,3-anhydro-D-mannose in pyridine after various times were then determined, with the results shown in Table 1. A notable feature was the behaviour of component A, which reached 80% of its equilibrium percentage after 3 h, whereas component B had then reached only 40% of its final value. In mutarotations in which more than two components are present in appreciable amounts the initial phase is concerned mainly with pyranose–furanose interconversion, while the later phase is dominated by pyranose–pyranose anomerisation [38-41] (ref. 41 contains a particularly clear analysis of the reactions involved). Component A is therefore probably a furanose form, (11) or (12), and it is especially interesting that it should be the major component of the equilibrium.

TABLE 1

Mutarotation of 2,3-anhydro-β-D-mannopyranose in pyridine; analysis of components A—D by g.l.c. of Tms ethers

	Time (min.)						
	2	5	15	45	90	180	1140 and 2580
% D	96·6	93·3	91·1	77·5	63·8	39·6	6·7
% C						3·3	5·0
% B	2·2	2·0	2·1	4·3	7·3	13·4	33·0
% A	1·2	4·7	6·8	18·2	28·9	43·7	55·3

Combined g.l.c.–mass spectrometry (g.l.c.–m.s.) has been used to analyse the products resulting from the trimethylsilylation of equilibrium mixtures of D-galactose and D-glucose.[42] We have now used it to determine the ring structures of the individual components A—D in the mixture of Tms ethers derived from 2,3-anhydro-D-mannose. Under conditions of g.l.c.–m.s. no separation of peaks B and C was observed, and the mass spectra of material at the beginning and at the end of this peak were recorded. It was considered that material at the beginning of this peak should be B and at the end a mixture of B and C, but the spectrum of the latter closely resembled that of B and no information on the structure of C could be obtained from it. The spectra of components B and D were very similar and different from that of A. In particular, the peak at *m/e* 205 had 60% relative abundance in the case of A, but only 10 and 5% for B and D, respectively. This peak can be attributed in A to the fragment (16) containing C-5 and C-6 formed by cleavage of the bond between C-5 and the furanose ring with charge retention on the former.[42] Other fragments which could be identified as belonging to the pyranose form of the sugar are as follows. A fragment at *m/e* 246 can be explained by the loss of C-5,C-6 and ring oxygen from the pyranose molecular ion (17) to give (18).[43] This ion had relative abundances of 20 and 8% for B and D, respectively, and only 3% for A. A fragment of *m/e* 217 may be (19), arising as shown.[43] The relative abundances for B and D were 35 and 27%, respectively, to be compared with 12% for A. This evidence derived from g.l.c.–m.s. supported the assignment of structure (9) to the crystalline 2,3-anhydro-D-mannose, and allows the assignment of structure (10) (2,3-anhydro-α-D-mannopyranose) to the component whose Tms ether is responsible for peak B. The earlier conclusion that the major component of the equilibrium mixture was a furanose, (11) or (12), corresponding to peak A after conversion into its Tms ether, was confirmed.

The equilibrium mixture was also examined by n.m.r. spectroscopy. Previous work on related 2,3-anhydropyranosides and -furanosides has shown that generally the coupling between H-1 and H-2 is negligible and thus the H-1 signal appears as a singlet. 2,3-Anhydro-β-D-

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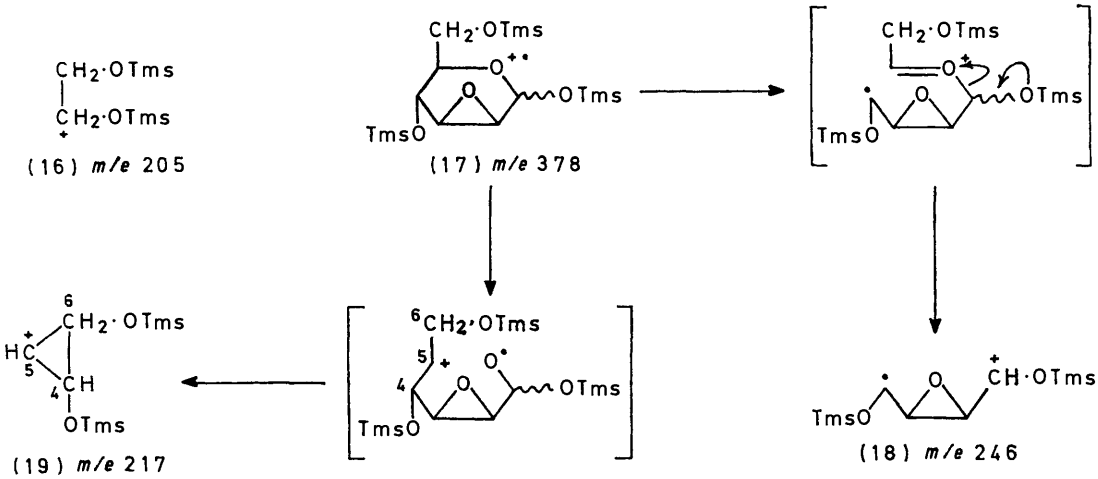
mannopyranosides,[32,44] including the benzyl glycoside (7), show the H-1 signal as a singlet. In the α-pyranoside series, on the other hand, low coupling constants in the region 0·66—0·74 Hz have been found for 2,3-anhydro-derivatives of mannose, rhamnose, and lyxose.[45] The spectra of both anomers of ethyl 2,3-anhydro-D-lyxo-furanoside in chloroform solution show the H-1 signal as a sharp singlet.[46]

The n.m.r. spectrum of an equilibrium mixture of 2,3-anhydro-D-mannose in deuterium oxide showed signals at τ 4·62 (s), 4·64 (d, $J_{1,2}$ 0·5 Hz), and 4·74 (s) for H-1, in the ratio 67 : 25 : 8. The doublet of low coupling constant is probably due to H-1 in the α-pyranose (10), in keeping with the percentage of this isomer derived from g.l.c. and g.l.c.–m.s. The H-1 signal at τ 4·74 is probably due to the β-pyranose (9) and the lowest field signal (τ 4·62) to the major furanose anomer (11) or (12). It was not possible to confirm this by direct measurement of the n.m.r. spectrum of the crystalline isomer (9) in deuterium oxide because of its rapid mutarotation. Mutarotation is slow, however, in [$^2H_6$]dimethyl sulphoxide,[39,47-50] and the n.m.r. spectrum of the pure isomer could be observed. The low-field signal due to OH-1 appeared at τ 3·26, showing strong coupling to H-1 (J 7·0 Hz), in complete agreement with the β-pyranose structure.[47,49] A drop of deuterium oxide was added to the solution and the isomerisation studied. The n.m.r. spectrum taken immediately after addition no longer showed the low-field signals due to OH-1, OH-4, and OH-6, and the signal for H-1 had collapsed to a singlet at τ 4·98. Two new signals at τ 4·82 and 4·84 had also appeared, and after 1 h the new signal at τ 4·82 was about the same size as the original signal at τ 4·98. After 20 h the three signals at τ 4·82, 4·84, and 4·98 were present in the ratio 63 : 32 : 5, and there was no further change with time, thus confirming the earlier results.

The fact that the major component of the equilibrium mixture is a furanose, (11) or (12), is of great interest. In related systems it has been found that 2,3-*O*-isopropylidene-L-rhamnose [50,51] exists in the furanose form to the extent of 65% in aqueous solution and that D-mannose 2,3-carbonate is almost completely in the furanose form.[51] In dimethyl sulphoxide both D-mannose 2,3-carbonate and D-lyxose 2,3-carbonate are in the furanose form.[48] A six-membered ring is distorted when fused to a five-membered ring and the pyranose ring form is therefore strained.[40,50] The arrangement of two *cis*-1,2-fused five-membered rings is relatively free from steric strain,[52] resulting in a strong preference for the furanose ring in the sugar portion. It appears that the oxiran ring has a similar effect in giving a more stable arrangement when fused to a five-membered ring.



SCHEME 2

The anomeric configuration of the preferred furanose form remained to be determined. In the case of 2,3-*O*-isopropylidene-L-rhamnofuranose Angyal argues that the β-isomer should be much less stable than the α, because of the presence of all the substituents *cis* on a five-membered ring.[50] Similar arguments should apply in the present case, implying that the major component of the 2,3-anhydro-D-mannose equilibrium mixture is the α-furanose (12), and experimental verification of this by polarimetry was sought.

If r_t is the rotation at time t and r_∞ the equilibrium rotation, the plot of log $(r_t - r_\infty)$ against t is linear when only two components are present in the mutarotational equilibrium, and the mutarotation is described as ' simple '.[39] When more than two components are present the graph is usually non-linear and the mutarotation is ' complex '. The mutarotation of 2,3-anhydro-β-D-mannopyranose in water was rapid at room temperature and equilibrium was reached ($[\alpha]_D$ 0°) after *ca.* 1 h.

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The plot of log $(r_t - r_\infty)$ against t gave a straight line indicating that only two components were present. This was inconsistent with the g.l.c. and n.m.r. evidence, which clearly showed the presence of at least three species. Extrapolation of the straight line gave $[\alpha]_D^{25}$ +60° for the initial specific rotation of the crystalline anhydromannose (9).

We have made approximate calculations of the specific rotations of the four isomers of 2,3-anhydro-D-mannose (9)—(12), based on the values of the compounds in Table 2. In the calculation we have made the following assumptions: (i) that the molecular rotation, $[M]_D$, of a sugar differs by ±115° from that of the corresponding methyl glycoside;[41,53-55] (ii) that the molecular rotation of a 6-deoxyhexopyranose is 35° less than that of the corresponding hydroxymethyl compound:[53-55] (iii) that for the present purposes the asymmetry at C-5 in the furanoses (11) and (12) may be ignored and that direct comparison with 2,3-anhydro-D-lyxose derivatives is therefore valid. The difference in the calculated values for the specific rotations of the furanose isomers (11) and (12) did not enable us to differentiate between them by polarimetry. We therefore rely on the conformational arguments[50] that the preferred isomer is the α-furanose (12). If we make this assumption the calculated mutarotation is +66° ⟶ +5°, in good agreement with observation. We have noted with interest that the calculated rotation of the β-pyranose (9) is more positive than that of the α-pyranose (10). This may not be significant, however,

TABLE 2

Calculation of rotations of 2,3-anhydro-D-mannose [(9)—(12)]

Compound		$[\alpha]_D$(°) (in H_2O)	$[M]_D$(°)
Methyl 2,3-anhydro-6-deoxy-D-mannopyranoside	α	+102·7 [a]	+164
	β	−27·5 [b]	−44
Methyl 2,3-anhydro-D-lyxofuranoside	α	+57 [c]	+91
		+67 [d]	
	β	−106 [d]	−152
		−102 [e]	
2,3-Anhydro-D-mannopyranose	α(10)	+52 [f]	+84 [f]
	β (9)	+66 [f]	+106 [f]
2,3-Anhydro-D-mannofuranose	α (12)	−15 [f]	−24 [f]
	β (11)	−23 [f]	−37 [f]

[a] Ref. 45. [b] Ref. 32. [c] E. E. Percival and R. Zobrist, *J. Chem. Soc.*, 1953, 564. [d] B. R. Baker, R. E. Schaub, and J. H. Williams, *J. Amer. Chem. Soc.*, 1955, **77**, 7. [e] Dr. E. Percival, personal communication. [f] Calculated figure.

because if other values are used for converting the molecular rotations of glycosides into those of free sugars, *viz.* ±100° [54] or ±105°,[55] the calculated rotations change appreciably; when the value ±100° is used the calculated rotation of the α-isomer is the more positive.

A second route to 2,3-anhydro-D-mannose was developed, through the preparation of the benzyl glycoside in the α-series (13) by partial acidic hydrolysis of its 4,6-*O*-benzylidene derivative.[56] Hydrogenolysis yielded a mixture of isomers identical with those obtained from the β-glycoside (7). No crystalline product was obtained, but when the hydrogenation was carried out in tetrahydrofuran examination of the product by g.l.c. of the Tms ethers showed a high proportion of component B, as expected.

Hydrogenolysis of benzyl 3,4-anhydro-β-D-altropyranoside (8) in methanol gave 3,4-anhydro-D-altrose, (14) and (15), as a pure syrup, homogeneous by t.l.c. and paper chromatography. G.l.c. of the Tms derivative showed two components in the ratio 67·5 : 32·5 with retention times of 1·0 and 1·67 relative to the Tms ether of α-D-glucose. The n.m.r. spectrum of the anomeric mixture in deuterium oxide showed signals for H-1 at τ 5·14 ($J_{1,2}$ 4·3 Hz) and 5·26 ($J_{1,2}$ 1·7 Hz) in the ratio 35 : 65. It is very likely that both the α- and β-anomers will exist mainly in half-chair forms with the hydroxymethyl group pseudo-equatorial.[32,45] The α-anomer (14) will therefore have H-1 equatorial and its signal should appear at lower field than that due to the axial H-1 of the β-anomer (15). It is thus reasonable to assume that the signal at τ 5·14 belongs to H-1 of the α-anomer and the one at τ 5·26 to the β-anomer. Both constants $J_{1,2}$ are small, and it would be unsafe to assign configurations on these grounds alone. Nevertheless, the assignment of the β-structure to the major component is in agreement with $J_{1,2}$ for the β-glycoside (8) (1·5 Hz); methyl 3,4-anhydro-α-D-altropyranoside has $J_{1,2}$ 2·89 Hz.[45]

The 2,3-anhydro-D-mannose and 3,4-anhydro-D-altrose were tested as inhibitors of yeast hexokinase by Dr. E. M. Bessell and Professor A. B. Foster of the Chester Beatty Research Institute. Neither compound acted as an inhibitor under conditions where epoxypropyl β-D-glucopyranoside was active.[15] 3,4-Anhydro-D-altrose did, however, act as a substrate for the enzyme, albeit at high enzyme concentration.

EXPERIMENTAL

Light petroleum refers to the fraction b.p. 60—80°. Kieselgel G (Merck) was used as adsorbent for t.l.c.; carbohydrates were detected with anisaldehyde–sulphuric acid,[57] and toluene-*p*-sulphonates by treatment with alcoholic diphenylamine followed by exposure of the plate to u.v. light.[58] For column chromatography Silica Gel 7734 (70—325 mesh) ASTM (Merck) was used. In paper chromatography free sugars were detected by aniline phthalate [59] and epoxides by means of sodium iodide–Methyl Red; [33] R_{Glu} refers to rate of movement relative to glucose.

G.l.c. was performed using a Pye 104 gas chromatograph with a hydrogen flame ionisation detector [7 ft coiled glass column packed with 15% polyethylene glycol succinate on 100—120 mesh Diatomite C (Pye Unicam); column temperature 150°; nitrogen carrier gas flow rate of 40 ml min^{-1}]. Trimethylsilyl derivatives were prepared in the usual manner.[34] The percentage compositions of mixtures separated by g.l.c. was determined by cutting out and weighing the individual peaks from the chart. The combined g.l.c.–mass spectrometric studies were conducted

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with an A.E.I. MS30 spectrometer (ionising energy 70 eV), under the conditions already described.

Optical rotations were measured with a Bendix-NPL Automatic Polarimeter type 143 (1 cm tubes).

N.m.r. spectra were measured at 60 or 100 MHz with a Perkin-Elmer R-12 or Varian HA-100 spectrometer, respectively. Tetramethylsilane was used as internal standard for all solvents except deuterium oxide, for which sodium 3-(trimethylsilyl)propionate was used.

Methyl 2-O-p-*Tolylsulphonyl*-α-D-*glucopyranoside* (1).—Toluene-*p*-sulphonyl chloride (98·7 g, 1·4 mol. equiv.) was added to a solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside [60] (100 g) in anhydrous pyridine (200 ml) at 0—5° and the mixture was kept for 24 h at room temperature. The product was isolated with chloroform to give a syrup which crystallised with the addition of methanol to give a mixture of the mono- and bis-*O*-*p*-tolylsulphonyl derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (110 g).

The product (40 g) was added to methanol (400 ml) containing concentrated hydrochloric acid (8 ml) and the mixture was heated under reflux until t.l.c. (8 : 2 chloroform–ethyl acetate) showed that no starting material remained (5 h). The resulting solution was neutralised with lead carbonate and filtered, and the filtrate was evaporated under reduced pressure to give a syrup. T.l.c. of the syrup showed two components. The product crystallised out with the addition of ethanol and recrystallisation from ethyl acetate–light petroleum gave methyl 2-*O*-*p*-tolylsulphonyl-α-D-glucopyranoside [19 g, 46% from (1)], m.p. 137—138°, $[\alpha]_D$ +74° (*c* 0·5 in $CHCl_3$) {lit.,[24] m.p. 138—139°; lit.,[25] $[\alpha]_D$ +82·2° ($CHCl_3$)}.

1,3,4,6-*Tetra*-O-*acetyl*-2-O-p-*tolylsulphonyl*-α-(*and* β-)D-*glucopyranose* [(2) *and* (3)].—The sulphonate (1) (1 g) was added to a mixture of glacial acetic acid (3 ml), acetic anhydride (3 ml), and concentrated sulphuric acid (0·3 ml), and the mixture was stirred at 60° until t.l.c. (8 : 2 chloroform–ethyl acetate) showed that the triacetate of (1) (prepared in the normal manner) was no longer present (6 h). The mixture was then poured into aqueous sodium hydrogen carbonate and the product was extracted with diethyl ether. The extract was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a syrup that crystallised on addition of ethanol. Recrystallisation from ethanol gave the β-tetra-acetate (3) (0·18 g), m.p. 148—150°, $[\alpha]_D$ +17° (*c* 1·7 in $CHCl_3$) {lit.,[28] m.p. 159—160°, $[\alpha]_D$ +21° ($CHCl_3$)}.

The mother liquors from the crystallisation were evaporated under reduced pressure to give a syrup that crystallised spontaneously. Recrystallisation from ethanol gave the α-tetra-acetate (2) (0·73 g), m.p. 115—116°, $[\alpha]_D$ +73° (*c* 2·0 in $CHCl_3$) {lit.,[26] m.p. 127°, $[\alpha]_D$ +75° ($CHCl_3$)}.

3,4,6-*Tri*-O-*acetyl*-2-O-p-*tolylsulphonyl*-α-D-*glucopyranosyl Bromide* (4).—The glucopyranosyl bromide (4), m.p. 107—108° (lit.,[26] 113°), was prepared [27] from the acetate (2). An identical reaction with the acetate (3) gave the bromide (5).

Benzyl 3,4,6-*Tri*-O-*acetyl*-2-O-p-*tolylsulphonyl*-β-D-*glucopyranoside* (5).—To a solution of the bromide (4) (3 g) in benzyl alcohol (100 ml), anhydrous lead carbonate (3 g) and silver oxide (3 g) were added, and the mixture was stirred at room temperature. The reaction was followed by t.l.c. (9 : 1 chloroform–ethyl acetate) and after 24 h only a trace of the starting material remained. The situation had not altered after a further 24 h and the reaction was terminated at this point. The mixture was filtered through t.l.c. silica gel and the benzyl alcohol was removed by distillation (0·5 mmHg; bath temp. 90°). The resulting syrup was dissolved in diethyl ether and the solution filtered. Addition of light petroleum gave benzyl 3,4,6-tri-*O*-acetyl-2-*O*-*p*-sulphonyl-β-D-glucopyranoside (5) (2·12 g, 70%), m.p. 105—106, $[\alpha]_D$ −9° (*c* 2·0 in $CHCl_3$) {lit.,[30] m.p. 105—106°, $[\alpha]_D$ −7° ($CHCl_3$)}.

Benzyl 2,3-*Anhydro*-β-D-*mannopyranoside* (7) *and Benzyl* 3,4-*Anhydro*-β-D-*altropyranoside* (8).—Sodium methoxide [from sodium (0·85 g, 5 mol. equiv.) in methanol] was added to a solution of the sulphonate (5) (4 g) in anhydrous methanol (final volume 80 ml) and the solution was stirred at room temperature. After 0·5 h t.l.c. (6 : 3 : 1 light petroleum–ethyl acetate–ethanol) showed that no starting material remained, and that there were two compounds with similar R_F values. The slower-moving component gave a positive test for toluene-*p*-sulphonate. After 6 h t.l.c. showed that this slower-moving component was no longer present and that a third component of slightly higher R_F had appeared. The mixture was then neutralised with 0·5M-sulphuric acid and filtered, and the filtrate was evaporated under reduced pressure to give a syrup that crystallised spontaneously. Recrystallisation from ether–light petroleum gave *benzyl* 2,3-*anhydro*-β-D-*mannopyranoside* (7) (1·2 g, 65%), m.p. 128—129°, $[\alpha]_D$ −24° (*c* 1·0 in EtOH) (Found: C, 62·05; H, 6·5. $C_{13}H_{16}O_5$ requires C, 61·9; H, 6·35%); τ (100 MHz; [$^2H_5$]pyridine) 2·50—3·10 (6H, unresolved, Ph and OH), 3·50 (1H, s, OH), 4·78 (1H, s, H-1), 5·13 (2H, q, benzylic), 5·50—6·34 (4H, unresolved, H-4, H-5, and H-6), 6·38 (1H, d, *J* 4 Hz, H-2 or H-3), and 6·63 (1H, d, *J* 4 Hz, H-2 or H-3).

The mother liquors from the recrystallisation were placed on a column of silica gel (40 g) and eluted with 6 : 3 : 1 light petroleum–ethyl acetate–ethanol (25 ml fractions). Fractions 7 and 8, containing the faster-moving component (t.l.c.), were combined and evaporated under reduced pressure to give a syrup that crystallised spontaneously. Recrystallisation from ethyl acetate–light petroleum gave *benzyl* 3,4-*anhydro*-β-D-*altropyranoside* (8) (530 mg, 29%), m.p. 93—95°, $[\alpha]_D$ −77° (*c* 1·0 in EtOH) (Found: C, 62·05; H, 6·6%); τ (100 MHz; $CDCl_3$) 2·69 (5H, s, Ph), 5·31 (2H, q, benzylic), 5·43 (1H, d, $J_{1,2}$ 1·5 Hz, H-1), 5·80—6·55 (6H, unresolved, H-2, H-5, H-6, and OH), 6·61 (1H, q, H-3 or H-4), and 6·77 (1H, d, *J* 3·8 Hz, H-3 or H-4).

Benzyl 2,3-*Anhydro*-4,6-O-*benzylidene*-α-D-*mannopyranoside*.—Prepared by the method of Inch and Lewis,[56] the compound had m.p. 123—124°, $[\alpha]_D$ +90·5° (*c* 2·0 in $CHCl_3$) (lit.,[56] m.p. 118°). Dr. Inch has informed us that the value for the specific rotation given in ref. 56 is incorrect.

Benzyl 2,3-*Anhydro*-α-D-*mannopyranoside* (13).—The foregoing benzylidene acetal (0·5 g), suspended in 0·005M-sulphuric acid (25 ml) and methanol (25 ml) was heated under reflux until the solid had dissolved (3 h). T.l.c, (9 : 1 benzene–ether) showed the absence of starting material. The mixture was neutralised ($BaCO_3$) and filtered, and the filtrate was evaporated to yield a syrup which crystallised on treatment with light petroleum. Recrystallisation from ether–light petroleum gave the *epoxide* (13) (0·32 g, 86%), m.p. 71—72°, $[\alpha]_D$ +89° (*c* 1·0 in EtOH) (Found: C, 62·25; H, 6·3. $C_{13}H_{16}O_5$ requires: C, 61·9; H, 6·35%), τ (100 MHz; $CDCl_3$) 2·68 (5H, s, Ph), 4·94 (1H, s, H-1), 5·35 (2H, q, benzylic), 6·00—6·63 (5H, unresolved, H-4, H-5, H-6, and OH), 6·72 (1H, d, *J* 3·7 Hz,

[60] N. K. Richtmyer, *Methods Carbohydrate Chem.*, 1962, **1**, 107.

H-2 or H-3), 6·88 (1H, d, *J* 3·7 Hz, H-2 or H-3), and 7·12 (1H, s, OH).

2,3-*Anhydro*-D-*mannose*.—(*a*) To a solution of benzyl 2,3-anhydro-β-D-mannopyranoside (7) (0·5 g) in methanol (100 ml), 5% palladium–charcoal (0·4 g) was added, and the mixture was shaken at room temperature with a slight overpressure of hydrogen. After 90 min uptake was complete and the catalyst was removed. T.l.c. (6 : 3 : 1 light petroleum–ethyl acetate–ethanol) then showed a single spot just above the base line which was clearly different from that of a sample of the glycoside (7). The solution was evaporated under reduced pressure to give a syrup that crystallised spontaneously. Attempts to recrystallise this material were unsuccessful and subsequently it was no longer possible to obtain the product in a crystalline form. Paper chromatography (3 : 1 : 1 butan-1-ol–pyridine–water) of the syrupy product showed a single component (R_{Glu} 2·65) detected by either aniline phthalate or with the sodium iodide–Methyl Red reagent. Benzyl 2,3-anhydro-β-D-mannopyranoside (7) had R_{Glu} 3·99.

(*b*) In a separate experiment tetrahydrofuran was used as the solvent for the hydrogenolysis. Removal of catalyst followed by evaporation gave a crystalline residue which was isolated by trituration with acetone, followed by filtration. The crystals (0·16 g, 50%) were recrystallised from acetone to give the pure *epoxide* (9), m.p. 95—96°, $[\alpha]_D$ 0° (*c* 1·0 in H_2O; equilibrium) (Found: C, 44·65; H, 6·25. $C_6H_{10}O_5$ requires C, 44·45; H, 6·15%); τ [60 MHz; $(CD_3)_2SO$] 3·26 (1H, d, *J* 7·0 Hz, OH-1), 4·70 (1H, d, *J* 6·0 Hz, OH-4), 5·04 (1H, d, *J* 7·0 Hz, H-1), 5·46 (1H, t, *J* 5·8 Hz, OH-6), 6·1—6·8 (4H, unresolved, H-4, H-5, and H-6), 6·89 (1H, d, *J* 3·6 Hz, H-2 or H-3), and 6·95 (1H, d, *J* 3·6 Hz, H-2 or H-3).

(*c*) Benzyl 2,3-anhydro-α-D-mannopyranoside (13) was hydrogenolysed (2 h) as in (*b*), to give 2,3-anhydro-D-mannose as a syrup with chromatographic properties (paper chromatography, g.l.c.) identical with those described in (*a*) and (*b*).

(*d*) Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside was treated with 0·05M-sulphuric acid at 100° and samples were examined by paper chromatography.[20] A compound with the same chromatographic properties as the 2,3-anhydro-D-mannose prepared in (*a*)—(*c*) above was present in the early stages of the hydrolysis.

Mutarotation of 2,3-*Anhydro*-β-D-*mannopyranose* (9).—(*a*) The epoxide (9) (20 mg) was dissolved in water (1 ml) and immediately transferred to the 1 cm polarimeter cell. A plot of log $(r_t - r_\infty)$ against *t* gave a straight line, extrapolation of which to $t = 0$ gave $[\alpha]_D$ +60° (initial).

(*b*) The epoxide (9) (5 mg) was dissolved in anhydrous pyridine (0·5 ml) and after 2 min hexamethyldisilazane (0·1 ml) and chlorotrimethylsilane (0·05 ml) were added. The procedure was repeated with reaction times of 5, 15, 45, 90, 180, 1140, and 2580 min, and each sample was analysed by g.l.c. The results are shown in Table 1.

3,4-*Anhydro*-D-*altrose* [(14) *and* (15)].—The benzyl glycoside (8) (0·5 g) in methanol (50 ml) was hydrogenolysed similarly. 3,4-*Anhydro*-D-*altrose* was isolated as a colourless hygroscopic syrup, $[\alpha]_D$ +11° (*c* 1·0 in H_2O; equilibrium) (Found: C, 41·05; H, 6·45. $C_6H_{10}O_5,H_{1·5}O_{0·75}$ requires C, 41·05; H, 6·55%). Paper chromatography (butan-1-ol–pyridine–water, 3 : 1 : 1) showed a single component (R_{Glu} 2·54) detected by aniline phthalate or sodium iodide–Methyl Red. The 60 MHz n.m.r. spectrum (solvent D_2O) was very complex, but showed H-1 signals at τ 5·14 (d, $J_{1,2}$ 4·3 Hz) and 5·26 (d, $J_{1,2}$ 1·7 Hz) in the ratio 35 : 65.

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