6. *The Acid-catalysed Reversion of* L-*Arabinose and of* D-*Mannose.**

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The reversion by acids of L-arabinose and of D-mannose has been investigated. L-Arabinose is converted in part into 3- and 4-isomers of *O*-β-L-arabopyranosyl-L-arabinose; and β-L-arabopyranosyl β-L-arabopyranoside was separated from the mixture of oligosaccharides produced. D-Mannose yields a complex mixture of disaccharides and oligosaccharides from which 6-*O*-α- and 6-*O*-β-D-mannopyranosyl-D-mannose and (?) 4-*O*-β-D-mannopyranosyl-D-mannose have been isolated. The significance of these results is discussed.

The rate of hydrolysis of polysaccharides in solutions of dilute mineral acid depends mainly on the concentration of the acid and the temperature. Conversion of the polysaccharide into monosaccharide is never complete as the reaction is reversible and even under the most favourable conditions small amounts of oligosaccharide remain. Under less favourable conditions, that is high concentration of sugars, considerable quantities of oligosaccharide are formed in solution. One of the methods used to determine polysaccharide structure is to hydrolyse the polymer to smaller fragments which are then detected and separated chromatographically. In some cases the yields of oligosaccharides are very small and there is some doubt whether the isolated material is really part of the original polymer or whether it results from resynthesis (reversion) from smaller fragments which have been produced during the hydrolysis. As our interests have been in the chemistry of arabinose-, mannose-, and xylose-containing polymers the effect of acid on each of these three sugars has been studied. The object was to find which oligosaccharides are produced in conditions chosen such that a relatively large quantity of each monosaccharide is converted into oligosaccharides.

When L-arabinose was set aside in acid solution the optical rotation changed and oligosaccharides were readily detected chromatographically. It was very difficult to separate these oligosaccharides owing to the similarity of their chromatographic behaviour. However, after repeated fractionation on charcoal and cellulose columns, three disaccharides were separated and characterised. The major product was 3-*O*-β-L-arabopyranosyl-L-arabinose (A) which was conclusively identified. 4-*O*-β-L-Arabopyranosyl-L-arabinose (B) and *O*-β-L-arabopyranosyl β-L-arabopyranoside (C) were also separated and provisionally identified.

Disaccharide (A) was recognised by the formation of phenylosazone which was identical with that prepared from an authentic specimen. This identity was confirmed when the disaccharide was methylated and hydrolysed and the fragments were identified as 2 : 3 : 4-tri- and 2 : 4-di-*O*-methyl-L-arabinose.

Disaccharide (B), a chromatographically pure syrup, did not give a crystalline phenylosazone. Methylation gave a syrup which gave analytical figures of a hexamethyldipentoside and on hydrolysis furnished only 2 : 3 : 4-tri- and 2 : 3-di-*O*-methyl-L-arabinose. These were identified chromatographically and by the formation of crystalline derivatives of the corresponding acids. This evidence limits the structure of the disaccharide to structure (B) and 5-*O*-β-L-arabopyranosyl-L-arabofuranose.[2] Calculations of molecular rotation from Hudson's rule[3] show that structure (B) is preferred.

Disaccharide (C) can be separated chromatographically from the oligosaccharide mixture. It was obtained more readily when the mixture was boiled with aqueous sodium hydroxide, and the cooled solution then de-ionised on ion-exchange columns. This

* A preliminary account of this work was given in TAPPI, 1956, **39**, 438.

[1] Jones, *J.*, 1953, 1672.
[2] Cf. Stephen, *J.*, 1957, 1919.
[3] Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 537.

procedure destroyed all reducing sugars, the acidic decomposition products were absorbed, and the non-reducing disaccharide was found in the effluent from the column. It was characterised as the crystalline octa-acetate, the optical rotation (+232°) of which indicated that the two monosaccharide residues each possessed pyranose rings and β-glycosidic linkages. Calculations of molecular rotation by Hudson's rule confirm this view. It is of interest that the analogous α-linked trehalose-type disaccharide has been isolated after the action of sulphuric acid on D-arabinose.[5] The mixture of oligosaccharides produced on reversion of L-arabinose was very complex and contained several other di- and tri-saccharides which were not identified. If therefore hydrolysis of an arabinose-containing polymer produces only one disaccharide it is very probable that this is not formed by reversion, particularly if it is a disaccharide other than disaccharide (A). On the other hand, isolation of disaccharide (A) after hydrolysis of a polysaccharide, unless it is the only arabinobiose produced, may mean that reversion and synthesis of disaccharides has occurred.

When mannose is kept in 6N-hydrochloric acid the optical rotation of the solution rapidly becomes more positive and di- and oligo-saccharides can be detected on the chromatogram. The picture changes with time, some sugars, probably furanoside derivatives, which are formed initially, disappear and eventually an equilibrium of mono-, di-, tri-, and oligo-saccharides results. This equilibrium mixture was neutralised on an ion-exchange resin and the sugars were fractionated chromatographically, first on charcoal and then on cellulose columns. Four disaccharides were isolated and three were identified. These were (A) 6-*O*-α-D-, (B) 6-*O*-β-D-, (C) 3-*O*-α-D-, and (D) 4-*O*-β-D-mannopyranosyl-D-mannose.

Disaccharide (A) was obtained crystalline. Methylation followed by hydrolysis gave 2 : 3 : 4 : 6-tetra- and 2 : 3 : 4-tri-*O*-methyl-D-mannose which were characterised as the *N*-phenylmannosylamine [6] and as the derived lactone respectively.[7] This evidence proves that the sugars are linked either through position 5 or 6 of the reducing mannose moiety. Periodate oxidation of disaccharide (A) by Hough and Perry's method [8] yielded no formaldehyde, confirming this conclusion. Lead tetra-acetate oxidation [9] (Perlin, personal communication) proved that the disaccharide was the 1 : 6-derivative. Its optical rotation [3] indicates that the two mannose residues are joined by an α-glycosidic linkage.

The disaccharide (B) was a syrup which on methylation followed by hydrolysis gave a mixture identical with that isolated after hydrolysis of methylated disaccharide (A). On reduction it gave a syrupy mannitol derivative, one mole of which consumed six moles of periodate with the concomitant formation of four moles of formic acid, and one mole of formaldehyde, proving that compound (B) was the 1 : 6-β-linked disaccharide. Its optical rotation was in good agreement with the optical rotation calculated by Hudson's method.[10]

Component (C), 3-*O*-α-D-mannopyranosyl-D-mannose, was a syrup which on reduction with potassium borohydride, furnished a syrupy mannitol derivative, characterised as the crystalline deca-acetate. This mannitol derivative will also be produced when the 1 : 4-α-linked mannobiose is reduced and acetylated. Methylation of the reducing disaccharide gave an octamethyl derivative which on acidic hydrolysis yielded 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose, identified as described above, and crystalline 2 : 4 : 6-tri-*O*-methyl-D-mannose [11] identified by comparison with an authentic specimen. The optical

[4] Cf. Hough and Jones, *J.*, 1953, 344.
[5] Rice, *J. Amer. Chem. Soc.*, 1956, **78**, 6167.
[6] Irvine and McNicoll, *J.*, 1910, 1452.
[7] Haworth, Hirst, and Streight, *J.*, 1931, 1349.
[8] Hough and Perry, *Chem. and Ind.*, 1956, 768.
[9] Charlson and Perlin, *Canad. J. Chem.*, 1956, **34**, 1805, 1811.
[10] Hudson, *J. Amer. Chem. Soc.*, 1916, **38**, 1566.
[11] Haworth, Heath, and Peat, *J.*, 1941, 833.

rotation indicated that the mannose residues are α-linked. These results were confirmed by Perlin[9] (personal communication) who subjected the disaccharide to oxidation with lead tetra-acetate and obtained yields of formic acid and consumption of oxidant in agreement with theory.

Disaccharide (D) was isolated in small amount, and was not obtained chromatographically pure as it moved at the same rate as disaccharide (A) on cellulose columns. However, when a crude sample of compound (D) was methylated and hydrolysed, in addition to 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose, 2 : 3 : 4- and 2 : 3 : 6-tri-*O*-mannose were produced and detected chromatographically. Separation of the trimethyl fragments could be effected after the sugars had been converted by oxidation into the corresponding lactones. The identification of fraction (D) as a disaccharide rests on its rate of movement on the chromatogram.

The predominant sugar produced in this reversion was disaccharide (A), in which mannose residues are united through a primary alcohol group. This is to be expected because of the greater reactivity of that group. In addition, evidence was obtained of the presence of both a mannobiose of the trehalose type and a 1 : 6-mannosan. The experiment indicates that isolation of 1 : 6-linked mannose derivatives from a hydrolysate may result from reversion, but that if these are absent any other mannose disaccharides isolated are probably part of the structure of the polysaccharide which is undergoing hydrolysis. In this connection it will be noted that acidic hydrolysis of yeast mannan yielded 6-*O*-α-D-mannopyranosyl-D-mannose along with several other disaccharides. Acetolysis of yeast mannan [12] produced a different chromatographic picture, perhaps because the glycosidic linkage which is attached to a primary sugar-alcohol group is split more easily than those which are attached to secondary alcoholic groups. The two methods of hydrolysis may therefore be complementary. This isolation of the 1 : 6-linked mannobiose from yeast mannan cannot be taken as proving that 1 : 6-α-linked mannose residues are present in this polysaccharide.

It is concluded from this work and the accompanying paper that, in order to minimise the formation of acid reversion products, the hydrolysis of a polysaccharide shall be carried out in dilute solution, $\not>5\%$ and preferably 2%. Great care should be taken to see that the sugar solution is neutral before it is concentrated and that no acidity has developed during the concentration. The isolation of an oligosaccharide which contains 1 : 6-linkages should not be regarded as proof of its presence in a particular polysaccharide unless it is isolated in large yield. It is suggested that the true products of hydrolysis of the polymer may be determined by conducting the hydrolysis in the presence of radioactive monomers. All oligosaccharides from the polymer will then be non-radioactive.

Experimental

Solutions were concentrated under reduced pressure at 40° or lower. Chromatographic separations were conducted on charcoal columns,[13] cellulose columns,[14] and paper sheets. The charcoal columns were a mixture (1 : 1 w/w) of activated carbon (Darco G-60) and "Celite" and were packed as a slurry in water. The cellulose columns were packed as a slurry of Whatman cellulose powder in acetone which was immediately replaced by the solvent to be used in the separation. Paper separations were conducted on sheets of Whatman No. 1 and 3MM chromatographic paper. Optical rotations were determined on aqueous solutions and at 20° ± 2° unless otherwise stated.

Charcoal columns were irrigated with water and/or ethanol. Various irrigants were used for paper chromatography: (*a*) ethyl acetate–acetic acid–water (9 : 2 : 2); (*b*) butan-1-ol–pyridine–water (10 : 3 : 3); (*c*) butan-1-ol–ethanol–water (40 : 11 : 19), (c_1) (3 : 1 : 1); (*d*) benzene–ethanol–water (169 : 47 : 15); (*e*) ethyl methyl ketone–water (89 : 11) [15] (all v/v). In general

[12] Smith and Srivastava, *J. Amer. Chem. Soc.*, 1956, **78**, 1404.
[13] Whistler and Durso, *ibid.*, 1950, **72**, 677.
[14] Hough, Jones, and Wadman, *J.*, 1949, 2511.
[15] Partridge, *Biochem. J.*, 1948, **42**, 238.

solvent (*a*) was used to separate monosaccharides and certain of their derivatives, (*b*), (*c*), and (c_1) were used to separate disaccharides, and (*d*) and (*e*) to separate partially methylated monosaccharides. The eluates from cellulose columns were collected on an automatic fraction-collector.[14]

Sugars were detected on paper chromatograms by spraying with a 1% solution of *p*-anisidine hydrochloride [16] in butan-1-ol and heating (for reducing sugars) or with a 1% solution of silver nitrate in acetone followed by spraying with 2% ethanolic sodium hydroxide for non-reducing sugars.[17] Lactones were detected by the hydroxamic acid test.[18] The rates of movement of of sugars are quoted relative to a standard sugar, *e.g.*, R_{gal}, R_{Rh}, and R_G (relative to galactose, rhamnose, and 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose respectively).

Reversion of L-*Arabinose.*—L-Arabinose (76 g.) which contained a small amount of galactose (chromatographic evidence) was dissolved in 6N-hydrochloric acid (100 ml.), and the solution set aside for 96 hr. during which the change in optical rotation was followed: $[\alpha]_D$ +51·5° (0·75 hr.); 52·3° (2 hr.); 53·4° (4 hr.); 55·5° (8 hr.); 61·2° (24 hr.); 68·3° (48 hr.); 72·0° (96 hr.). The solution was then diluted and deacidified on Amberlite resin IR-4B(OH), and the neutral effluent was concentrated to a syrup. The syrupy product was then separated on a charcoal–Celite column [13] by passing water (fraction I), and then 5% ethanol–water (fraction II, III, and IV) through the column. The results are given in Table 1.

TABLE 1

Fraction	R_{gal} (irrigant *a*)	Wt. (g.)	Fraction	R_{gal} (irrigant *a*)	Wt. (g.)
I	Arabinose	33·7	III	1·2, 0·8, 0·5, 0·4, 0·2	5·2
II	Arabinose, 0·8, 0·4	25·6	IV	0·75, 0·5, 0·3	7·4
					71·9

Fraction II crystallised and the crystals were identified as L-arabinose; the residual syrup (13·2 g.) was almost free from L-arabinose. It was further fractionated on a cellulose column,[14] with butan-1-ol–water (10 : 1 v/v) as irrigant. When solvent (*b*) was used to examine the fraction obtained it was observed that the mixture was much more complex than was indicated by solvent system (*a*). Solvent (*b*) indicated the presence of components with R_{gal} 1·2, 0·72, 0·61, 0·55, 0·43, 0·36, 0·33, 0·28, and 0·23. In a similar fashion fractions III and IV were shown to be complex mixtures, and additional fractions were detected with R_{gal} 0·40 and 1·04.

After tedious further fractionation, first on cellulose columns and then on sheets of filter paper, a fraction (0·22 g.) with R_{gal} 0·76 (solvent *b*) was obtained chromatographically pure. It had $[\alpha]_D^{23}$ 192° (*c* 4·3) and when heated with phenylhydrazine acetate solution gave an osazone, m. p. 234°. This material was identical with the osazone of 3-*O*-β-L-arabopyranosyl-L-arabinose. A pure fraction with R_{gal} 0·55 (solvent *b*) was also isolated after a protracted series of fractionations. This substance (0·46 g.) had $[\alpha]_D$ 193° (*c* 5·0) and did not crystallise. A sample (0·35 g.) was methylated, first with sodium hydroxide and dimethyl sulphate and then with silver oxide and methyl iodide. The *product* had n_D^{21} 1·4710, b. p. 180° (bath-temp.)/0·05 mm., and $[\alpha]_D$ 128° (*c* 3·6 in $CHCl_3$) (Found: C, 52·7; H, 8·0; OMe, 49·2. $C_{16}H_{30}O_9$ requires C, 52·4; H, 8·3; OMe, 50·6%).

The syrupy material was hydrolysed by N-sulphuric acid at 93° for 4 hr. and neutralised with barium carbonate. The solution was filtered and concentrated to a syrup (0·29 g.) with $[\alpha]_D$ 136° (*c* 2·5), 64° (*c* 2·43 in $CHCl_3$). The material consisted of two sugars with R_{gal} 0·69 and 0·84 (solvent *b*) and R_{gal} 0·52 and 0·84 (solvent *d*). The mixture was separated on a sheet of filter paper (solvent *b*). The slower-moving fraction (0·06 g.) had $[\alpha]_D$ 92° (*c* 2·8) and was identical, chromatographically, with 2 : 3-di-*O*-methyl-L-arabinose. On oxidation by bromine water it yielded the well-characterised 2 : 3-di-*O*-methyl-L-arabonolactone, m. p. 35°, $[\alpha]_D$ −36° ⟶ −28° (10 days) (*c* 1·1). The faster-moving fraction (0·99 g.) had $[\alpha]_D$ 125° (*c* 4·7) and on oxidation with bromine water gave a lactone which when heated with alcoholic phenylhydrazine yielded 2 : 3 : 4-tri-*O*-methyl-L-arabonyl phenylhydrazide, m. p. 158°, identical with an authentic specimen.

When the disaccharide described above was acetylated there was obtained in very small yield an unidentified crystalline acetate, m. p. 167°.

[16] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[17] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[18] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.

Isolation of O-β-L-*Arabopyranosyl* β-L-*Arabopyranoside.*—L-Arabinose (16 g.) was dissolved in 6N-hydrochloric acid (20 ml.) and set aside at room temperature for 96 hr. Excess (25 ml.) of 10N-sodium hydroxide was added and the mixture was boiled under reflux for 5 hr. The cooled solution was de-ionised by Amberlite resins IR-120(H) and IR-4B(OH), and concentrated to a syrup (1·31 g.). The syrup was acetylated with pyridine (20 ml.) and acetic anhydride (8 ml.) for 48 hr. at room temperature and then poured into ice-water. The acetate was isolated by chloroform-extraction and slowly crystallised. The crystals, recrystallised from alcohol (yield, 0·02 g.), had m. p. 232°, $[\alpha]_D$ 232° (*c* 0·15 in $CHCl_3$) (Found: C, 49·6; H, 5·7. Calc. for $C_{22}H_{30}O_{15}$: C, 49·5; H, 5·6%).

Reversion of D-*Mannose.*—D-Mannose (75 g.) was dissolved in 6N-hydrochloric acid (125 ml.) and the solution was kept at room temperature for 168 hr., during which the change in optical rotation was: $[\alpha]_D$ +6·67° (initial); +7·09° (1 hr.); +8·77° (8 hr.); +9·46° (24 hr.); +9·88° (36 hr.). The solution was then diluted and deacidified by passing it down a column of Amberlite IR-4B(OH) resin. The neutral effluent was concentrated to a syrup and placed on a charcoal column (15 × 17 cm. diam.) and eluted with water (portions of 125 ml.). In this fashion seven fractions were obtained (see Table 2). When fractions 3 and 6 were refractionated on columns of cellulose the component with R_{gal} 0·39 (solvent c_1), *disaccharide* A, was obtained pure and crystalline. After recrystallisation from methanol–butan-1-ol it had m. p. 196—197°, $[\alpha]_D^{16}$ +52° (*c* 1·99) (Found: C, 42·2; H, 6·4. $C_{12}H_{22}O_{11}$ requires C, 42·1; H, 6·4%).

TABLE 2.

Fraction	R_{gal} (solvent c_1)	Wt. (g.)	Fraction	R_{gal} (solvent c_1)	Wt. (g.)
1	mannose	20·7	5	mannose, 0·54, 0·49, 0·39	0·7
2	mannose, 0·54, 0·39	19·9	6	0·54, 0·49, 0·49 high mol. wt.	10·7
3	2·6, 0·54, 0·39	10·3	7	0·54, high mol. wt.	10·9
4	0·39	1·9			75·1

Methylation of the Disaccharide A.—A portion (0·82 g.) of the crystalline reducing disaccharide was methylated with sodium hydroxide and methyl sulphate in the usual way. The *product* (0·58 g.), n_D^{18} 1·4640, distilled at b. p. 192° (bath-temp.)/0·1 mm. The distillate (0·40 g.) had $[\alpha]_D$ +58° (*c* 3·74 in $CHCl_3$) (Found: C, 52·8; H, 8·3; OMe, 54·4. $C_{20}H_{38}O_{11}$ requires C, 52·9; H, 8·4; OMe, 54·6%). The methylated product (0·34 g.) was hydrolysed in N-sulphuric acid (15 ml.) at 100° for 162 hr., during which the optical rotation changed from +40° to +6°. The solution was neutralised with barium carbonate and concentrated to a syrup (0·3 g.) which was fractionated on sheets of Whatman No. 3MM filter paper. The slower-moving component, with R_F 0·55 (0·09 g.), had $[\alpha]_D$ +3° (*c* 0·37) (Found: OMe, 41·8. Calc. for $C_9H_{18}O_6$: OMe, 41·9%.) It was oxidised with bromine water and yielded 2 : 3 : 4-tri-*O*-methyl-D-mannonolactone, which moved at the same rate on the chromatogram as an authentic specimen (R_F 0·75, solvent c_1, hydroxamic acid spray) and had m. p. and mixed m. p. 72—73°, $[\alpha]_D$ 136° ⟶ +82° (85 hr.) (*c* 0·28). The *faster-moving component* (0·12 g.), with R_F 0·78 (solvent *e*), had $[\alpha]_D$ 2·9° (*c* 0·45). When this syrup was heated with alcoholic aniline the *N*-phenylglycosylamine 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose resulted, with m. p. 164—165° which fell to 144—145° after recrystallisation from ethanol, $[\alpha]_D$ 95° ⟶ −7° (137 hr.; *c* 0·59 in MeOH) (Found: C, 61·6; H, 8·1; N, 4·7. Calc. for $C_{16}H_{25}O_5N$: C, 61·7; H, 8·0; N, 4·5%).

The component in fraction 3, with R_{gal} 2·6 (solvent c_1), was non-reducing and was detected with the silver nitrate spray. It was present in small amount and had $[\alpha]_D$ −79° (*c* 0·29). It was not identified but may be 1 : 6-anhydro-β-*O*-D-mannopyranose.

The disaccharide with R_{gal} 0·54 (solvent c_1), disaccharide C, was obtained pure by refractionation, on a cellulose column, of fraction 3. The substance (1·43 g.) had $[\alpha]_D$ +50° (*c* 2·86).

Methylation of Disaccharide C.—A portion of the reducing disaccharide (0·72 g.) was methylated in the usual way with methyl sulphate and sodium hydroxide and then with silver oxide and methyl iodide. The product (0·7 g.) distilled at 197° (bath-temp.)/0·3 mm. as a syrup (0·48 g.) (Found: C, 53·0; H, 8·3; OMe, 54·8. $C_{20}H_{38}O_{11}$ requires C, 52·9; H, 8·4; OMe, 54·6%). The methylated *product* (0·47 g.) was hydrolysed with 45% formic acid (25 ml.) at 100° for 7 hr., $[\alpha]_D$ 64° falling to 19° (const.). The solution was evaporated to a syrup which was heated for 1 hr. with N-sulphuric acid, in order to hydrolyse formyl esters. The solution was then extracted continuously with chloroform and the extracts were concentrated to a syrup (0·46 g.). The mixture was separated chromatographically (solvent *e*). The slower-moving of the components (0·15 g.) crystallised and after recrystallisation from acetone–light

petroleum had m. p. 64—65°, unchanged on admixture with 2 : 4 : 6-tri-*O*-methyl-D-mannose, and $[\alpha]_D$ + 16·5° (*c* 3·0). The faster-moving component (0·17 g.) had $[\alpha]_D$ +3° (*c* 1·7) and with ethanolic aniline gave the *N*-phenylglycosylamine, m. p. 144°, of 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose.

Reduction.—A portion of the reducing disaccharide (0·20 g.) was dissolved in water and reduced by the portionwise addition of an excess of potassium borohydride. The solution was acidified with acetic acid and de-ionised on Amberlite resin IR-120 and IR-4B. The neutral solution was evaporated to the syrupy mannitol derivative (0·17 g.), R_{ga} 0·37 (solvent *a*), $[\alpha]_D$ +59° (*c* 1·7 in MeOH). The syrup was acetylated in pyridine (5 ml.) and acetic anhydride (2 ml.) at 20° for 30 hr. The *product* (0·35 g.) had m. p. 116° (from ethanol), $[\alpha]_D$ 47° (*c* 2·2 in acetone) (Found: C, 50·1; H, 5·7. $C_{30}H_{42}O_{20}$ requires C, 49·9; H, 5·8%).

Fraction 6 was further fractionated on several sheets of Whatman No. 3MM paper and gave two chromatographically pure disaccharides (R_{gal} 0·39 and 0·51). The disaccharide fraction B, with R_{gal} 0·39 (0·5 g.), had $[\alpha]_D$ −12·4° (*c* 1·8), and gave on hydrolysis only D-mannose. A portion (0·1 g.), on reduction of its aqueous solution with potassium borohydride, gave the syrupy mannitol derivative (0·098 g.), $[\alpha]_D$ −21° (*c* 1·0).

The mannitol derivative (0·098 g.) was oxidised with 0·06M-sodium metaperiodate (100 ml.), and the solution was analysed at intervals for formic acid produced and metaperiodate consumed. After 4 hr. four moles of formic acid had been produced and 6 moles of periodate reduced per mole.

Methylation of Disaccharide B.—The reducing disaccharide (0·25 g.) was methylated, first with sodium hydroxide and methyl sulphate and then with silver oxide and methyl iodide. The pure *product* (0·21 g.) had n_D^{21} 1·4621, $[\alpha]_D$ −26° (*c* 2·1 in $CHCl_3$) (Found: OMe, 54·7. $C_{20}H_{38}O_{11}$ requires OMe, 54·6%). It was hydrolysed by heating its solution in N-sulphuric acid (25 ml.) at 98° for 7 hr., $[\alpha]_D$ −19° ⟶ +3°. When hydrolysis was complete the solution was extracted continuously with chloroform and the extracts were concentrated to a syrup (0·19 g.). The methylated components were separated (solvent *d*) by filter-paper chromatography. The tri-*O*-methyl fraction (0·05 g.) had $[\alpha]_D$ 1·6° (*c* 2·5). Oxidation with bromine water converted it into 2 : 3 : 4-tri-*O*-methyl-D-mannonolactone, m. p. and mixed m. p. 72—73°, $[\alpha]_D^{23}$ 130° ⟶ 81° (90 hr.). The tetra-*O*-methyl-D-mannose fraction (0·08 g.) was characterised as 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-mannosylamine, m. p. 145°.

Methylation of Disaccharide D.—The substance with R_{gal} 0·51 (solvent c_1) (0·2 g.), $[\alpha]_D$ −8·5° (*c* 2·0), was methylated with methyl sulphate and sodium hydroxide and then with silver oxide and methyl iodide. The methylated *product* (0·15 g.) had $[\alpha]_D$ −12° (*c* 3·1 in $CHCl_3$) and n_D^{20} 1·4640 (Found: OMe, 54·1. $C_{20}H_{38}O_{11}$ requires OMe, 54·6%). The methylated material was hydrolysed by N-sulphuric acid (15 ml.) at 95°, $[\alpha]_D$ −17° changing to +18° (7 hr.). The hydrolysis mixture was extracted continuously with chloroform, and the extracts were concentrated to a syrup (0·13 g.), which was separated on sheets of Whatman No. 1 filter paper. The tetra-*O*-methyl fraction (0·05 g.) was characterised as the crystalline *N*-phenylmannosylamine derivative, m. p. 145°. The tri-*O*-methyl fraction (0·09 g.) was a mixture which could not be separated chromatographically. Accordingly it was oxidised (bromine water) and the lactones were isolated. These crystallised in part. Paper-chromatographic separation of the mixture indicated (tentatively) the presence of the 2 : 3 : 6- and 2 : 3 : 4-tri-*O*-methyl isomers of D-mannonolactone (detected by the hydroxylamine spray).

Hydrolysis of Yeast Mannan.—Yeast mannan (2 g.) was hydrolysed in 0·5N-sulphuric acid (50 ml.) for 10 hr. at 100°. The hydrolysis mixture was neutralised with barium carbonate, filtered, and evaporated to a syrup (1·83 g.). Paper chromatography of its solution (solvent c_1) indicated the presence of mannose and four disaccharides with R_{gal} 0·58, 0·51, 0·42, and 0·36, the third disaccharide predominating. The syrup was fractionated on filter paper and the portions with R_{gal} 0·42 isolated. This material crystallised and was recrystallised from methanol–butan-1-ol. The substance (0·18 g.) had m. p. 196—197°, not depressed on admixture with 6-*O*-α-D-mannopyranosyl-D-mannose. It had $[\alpha]_D$ +53° (*c* 1·8) (Found: C, 42·1; H, 6·2. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·4%). When D-mannose (1·8 g.) was heated in 0·5N-sulphuric acid (50 ml.) at 100° for 10 hr. it was converted in part into a mixture of oligosaccharides. The solution was neutralised as above, filtered, and concentrated. Chromatography of the syrup gave a picture very similar to that produced when hydrolysed yeast mannan was examined chromatographically.

Acetolysis of Yeast Mannan.[12]—Yeast mannan (2·3 g.) was added in small portions to a

well-stirred mixture of acetic anhydride (8·8 ml.) and sulphuric acid (0·9 ml.) at 0°. After 2 hr. at 0° the solution was stirred at 20° for 12 hr. Acetic anhydride (2·3 ml.) was then added and stirring continued for a further 33 hr. at 20° and then at 90° for 15 min. The cooled mixture was poured on ice, and the acetylated product collected. The white powder (2·4 g.) was deacetylated catalytically [19] and the product examined chromatographically. Mannose, a disaccharide, and a trisaccharide were detected. The disaccharide had R_{gal} 0·59 (solvent c_1); the disaccharide with R_{gal} 0·42 was present in traces only.

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