46. *The Mannans of Ivory Nut* (Phytelephas macrocarpa). *Part II.*[1] *The Partial Acid Hydrolysis of Mannans A and B.*

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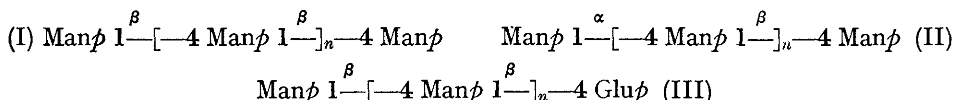
Partial acetolysis of mannan A, followed by deacetylation, afforded a homologous series of β-1 : 4-linked oligosaccharides (mannobiose to mannopentaose). Smaller quantities of mannose-containing oligosaccharides with some α-1 : 4-linkages, and of β-1 : 4-linked oligosaccharides containing both mannose and glucose residues were isolated. A similar series of oligosaccharides was isolated from mannan B. The significance of these results is discussed.

It was shown in Part I [1] that ivory nut mannans A and B are both mixtures of at least two molecular species, terminated by D-mannopyranose and D-galactopyranose residues; both species are linear, but in addition to the dominant β-1 : 4-mannopyranosyl linkages, the evidence indicated the presence also of some mannose residues linked through $C_{(1)}$ and $C_{(6)}$. In order to obtain further information concerning the detailed molecular structure of these polysaccharides we have studied the products of partial acid hydrolysis.

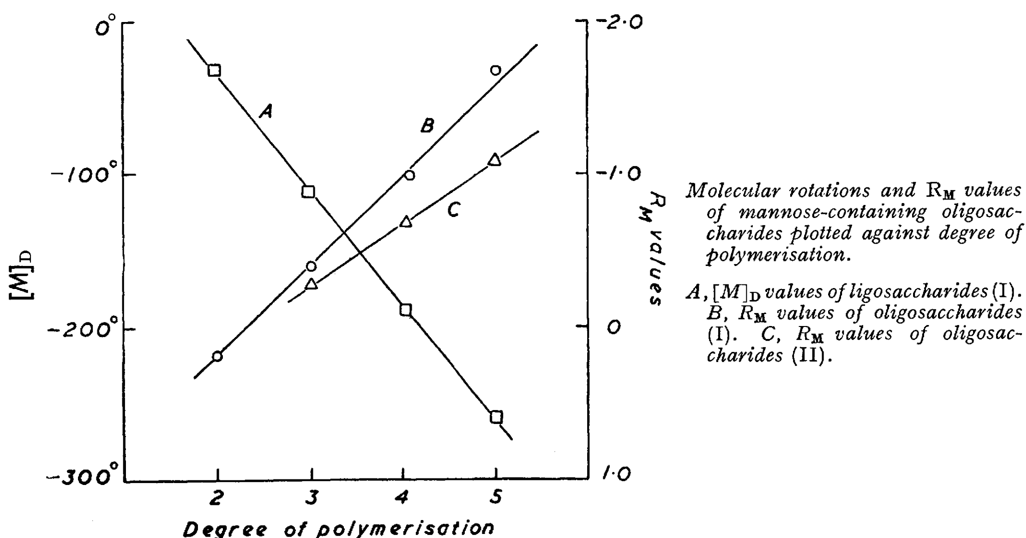
Since the mannans are relatively insoluble in water, partial acid hydrolysis was most conveniently effected by acetolysis with a mixture of acetic anhydride, acetic acid, and sulphuric acid; the products thereof were deacetylated with barium methoxide, and the resulting mixtures were fractionated by chromatography on charcoal, followed where necessary by partition chromatography on cellulose. Thus mannan A afforded as the main products a homologous series of β-1 : 4-linked oligosaccharides composed solely of D-mannose residues. Mannobiose gave an identical *X*-ray powder photograph and had a similar melting point, but differed slightly in optical rotation from 4-*O*-β-D-mannopyranosyl-D-mannose (I; $n = 0$) isolated by Whistler and Stein from the partial acid hydrolysis of guaran.[2] The structure of the disaccharide was confirmed in studies of

[1] Part I, Aspinall, Hirst, Percival, and Williamson, *J.*, 1953, 3184.
[2] Whistler and Stein, *J. Amer. Chem. Soc.*, 1951, **73**, 4187.

methylation and periodate oxidation. Mannotriose crystallised as the trihydrate and gave an identical *X*-ray powder photograph and had a similar melting point, but again differed slightly in optical rotation from the β-1 : 4-linked mannotriose (I; $n = 1$) previously isolated from the partial acid hydrolysis of guaran.[3] The derived mannotri-itol dodeca-acetate had similar physical constants to those quoted by Whistler and Smith.[3] Mannotetraose (I; $n = 2$) and mannopentaose (I; $n = 3$) were established as members of the same homologous series by the following observations: (i) the molecular rotations and the R_M values[4] of the sugars, mannobiose to mannopentaose, when plotted against degree of polymerisation gave straight line graphs (see Figure); (ii) chromatographic examination of the products of partial acid hydrolysis showed mannose and the lower homologues of the series.



Small quantities of two other series of oligosaccharides were also isolated; the first series contained only mannose residues, but in the second series each oligosaccharide



Molecular rotations and R_M *values of mannose-containing oligosaccharides plotted against degree of polymerisation.*

A, $[M]_D$ *values of ligosaccharides* (I). *B*, R_M *values of oligosaccharides* (I). *C*, R_M *values of oligosaccharides* (II).

contained mannose residues and one glucose residue per molecule. Disaccharide A gave only mannose on hydrolysis and had an optical rotation indicative of an α-glycosidic linkage. The following observations leave little doubt that this disaccharide is 4-*O*-α-D-mannopyranosyl-D-mannose (II; $n = 0$): (i) periodate oxidation[5] afforded formaldehyde, indicating the absence of a 1 : 5- or 1 : 6-linkage; (ii) reaction with phenylhydrazine gave a disaccharide phenylosazone, showing that a 1 : 2-linkage was not present; (iii) chromatography showed the presence of a tetrose (erythrose) when the disaccharide was oxidised with lead tetra-acetate under controlled conditions and the product hydrolysed,[6] indicating the presence of a 1 : 4-linked disaccharide (under similar conditions 1 : 3-linked hexose-containing disaccharides yield pentoses). Confirmation of the presence of the α-1 : 4-linkage in the disaccharide came from experiments carried out on trisaccharide C.

[3] Whistler and Smith, *J. Amer. Chem. Soc.*, 1952, **74**, 3795.
[4] Bate-Smith and Westhall, *Biochem. Biophys. Acta*, 1950, **4**, 427.
[5] Reeves, *J. Amer. Chem. Soc.*, 1941, **63**, 1476.
[6] Perlin, *Analyt. Chem.*, 1955, **27**, 396.

Hydrolysis of the crystalline trisaccharide gave only mannose, and partial acid hydrolysis gave both mannobioses and mannose, but partial acid hydrolysis of the derived glycitol (potassium borohydride reduction) gave only the α-linked mannobiose and mannose. When oxidised with periodate the quantity of reagent consumed by the trisaccharide was exactly parallel to the quantity consumed by the β-linked mannotriose (I; $n = 1$), suggesting the presence of similar linkages in the two trisaccharides. The optical rotation of trisaccharide C was consistent with the presence of one α-linked and one β-linked D-mannopyranosyl residue. Proof that the non-reducing end-group (with the α-configuration) was glycosidically linked to position 4 of the central mannose unit in the trisaccharide came from the isolation of 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methyl-D-mannose from the hydrolysis of the methylated glycitol. Trisaccharide C is, therefore, *O*-α-D-mannopyranosyl-(1 ⟶ 4)-*O*-β-D-mannopyranosyl-(1 ⟵ 4)-D-mannopyranose (II; $n = 1$). Small quantities of a second tetrasaccharide and a second pentasaccharide were also isolated. It is probable from chromatographic examination of the oligosaccharides and their partial hydrolysis products that these belong to the same homologous series (II; $n = 2$ and 3).

The glucose-containing oligosaccharides were not isolated in sufficient quantity for complete characterisations to be carried out. The following observations, however, suggest that the disaccharide B is probably 4-*O*-β-D-mannopyranosyl-D-glucose (III; $n = 0$): (i) hydrolysis of the disaccharide gave glucose and mannose, but hydrolysis of the derived aldobionic acid (bromine oxidation) gave only mannose; (ii) the optical rotation indicated that the mannosylglucose was β-linked; (iii) periodate oxidation [5] afforded formaldehyde, indicating the absence of a 1 : 5- or 1 : 6-linkage; (iv) reaction with phenylhydrazine gave a disaccharide phenylosazone, showing that a 1 : 2-linkage was not present; (v) controlled oxidation of the disaccharide with lead tetra-acetate followed by hydrolysis of the oxidation product gave erythrose, pointing to a 1 : 4-linkage. Since the phenylosazone of disaccharide B was different from that derived from β-1 : 4-linked mannobiose (I; $n = 0$), there is still doubt concerning the structure of the disaccharide. Trisaccharide D, which gave on hydrolysis mannose and glucose in the approximate ratio of 2 : 1, had an optical rotation indicative of β-linkages. Partial acid hydrolysis of the trisaccharide gave mannose, glucose, mannobiose (I; $n = 0$), and disaccharide A, but on hydrolysis of the derived glycitol only mannose and mannobiose could be detected. Subject to the same reservations as in the case of disaccharide B, the trisaccharide probably belongs to the same homologous series (III; $n = 1$).

The partial acid hydrolysis of mannan B afforded a similar series of oligosaccharides. Again, the major products were the β-1 : 4-linked oligosaccharides (I; $n = 0$, 1, and 2), which were isolated as the crystalline sugars. The other oligosaccharides (II; $n = 0$ and 1) and (III; $n = 0$ and 1) which had been isolated from mannan A were identified chromatographically.

These results provide additional evidence for the presence in the ivory nut mannans of chains of 1 : 4-linked β-D-mannopyranosyl units, and again show that there are no essential structural differences between mannans A and B. The presence, however, of a small proportion of α-linked D-mannose units was not previously suspected. From the present evidence it is probable that the α-linked oligosaccharides are of structural significance and are not artefacts, since control experiments provided no evidence for their formation either from mannose by reversion or from the β-linked oligosaccharides by acid-catalysed anomerisation at the glycosidic bond.[7] It is not yet possible to assess the structural significance of the glucose-containing oligosaccharides. Although the ivory nut mannans contain a small proportion of glucose residues so that partial hydrolysis should give rise to some glucose-containing oligosaccharides, these substances may have been formed by epimerisation of the mannose-containing oligosaccharides. No further evidence was found to confirm the presence in the mannans of 1 : 6-linkages.[1]

[7] Cf. Lindberg, *Acta Chem. Scand.*, 1949, **3**, 1153.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) ethyl acetate–pyridine–water (10 : 4 : 3); (B) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (C) butan-2-one saturated with water; (D) ethyl acetate–propan-2-ol–water (16 : 10 : 5).

Mannan A was prepared by extraction of delignified ivory nut shavings with 7% aqueous potassium hydroxide as described previously.[1] The polysaccharide, which had $[\alpha]_D^{18}$ $-48°$ (*c* 1·1 in N-NaOH), was used without further purification. Mannan B was prepared as described previously [1] and had $[\alpha]_D^{18}$ $-26°$ (*c* 1·0 in anhydrous formic acid).

Acetolysis of Mannan A and Fractionation of Derived Oligosaccharides.—Mannan A (55 g.) was added slowly with stirring to a mixture of acetic anhydride (330 ml.), glacial acetic acid (330 ml.), and concentrated sulphuric acid (33 ml.) at 0°. The mixture was kept at room temperature for 72 hr., during which the mannan had completely dissolved (*ca.* 36 hr.). The mixture was filtered, then poured slowly with stirring into ice-water, and sodium hydrogen carbonate was added gradually (to pH 3—4). The precipitated sugar acetates were filtered off, and the filtrate was extracted with chloroform (3 × 800 ml.). The solid acetates were dissolved in chloroform, the solution was combined with the chloroform extracts, and the combined solutions were washed with sodium hydrogen carbonate solution, dried, and concentrated. A solution of barium methoxide (7·5 g.) in methanol (75 ml.) was added to a solution of the sugar acetates (90·7 g.) in chloroform (250 ml.) and methanol (500 ml.) at 0°, and the mixture was shaken for 1 hr. and set aside overnight at 0°. The mixture was exactly neutralised by the addition of dilute sulphuric acid, and water (175 ml.) was added. The chloroform layer was separated, and the aqueous layer was filtered through a pad of " Celite " and concentrated. The mixture of sugars (45·8 g.), dissolved in water (200 ml.), was added to a column of charcoal–Celite (1·3 kg.; 2 : 1). Elution with water and water containing 0·5—4·0% of ethanol yielded a mixture of monosaccharides (mannose, together with small quantities of xylose, glucose, and galactose) (6·5 g.) which was not examined further. Oligosaccharides were eluted with ethanol–water containing increasing proportions of ethanol and eight fractions were collected.

Examination of Oligosaccharide-containing Fractions.—*Fraction* 1. The sugar (9·8 g.; eluted with water containing 4·5—6·5% of ethanol) was crystallised from ethanol–water and had $R_{mannose}$ 0·52 in solvent A, m. p. 202—203°, and $[\alpha]_D^{19}$ $-5·2° \longrightarrow -8·5°$ (2 hr., const.) (*c* 5·4 in H_2O). Whistler and Stein [2] report m. p. 193·5—194° and $[\alpha]_D^{25}$ $-7·7° \longrightarrow -2·2°$ (equil.) (in H_2O) for 4-*O*-β-D-mannopyranosyl-D-mannopyranose, but the two samples gave identical *X*-ray powder photographs (comparison kindly undertaken by Professor R. L. Whistler). Treatment of a sample of the sugar with sodium metaperiodate solution [8] resulted in the consumption of 4·0 mol. of reagent after 48 hr., followed the consumption of a fifth mol. after 120 hr.; further reaction occurred slowly thereafter. On reaction with potassium metaperiodate [8] 3 mol. of formic acid were released after 185 hr., but no definite break in reaction occurred at this point. A sample (*ca.* 1 g.) of the sugar was methylated successively with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide, to yield the fully methylated disaccharide (310 mg.). A portion of the methylated sugar (100 mg.) was hydrolysed by N-sulphuric acid in a sealed tube at 100° for 6 hr. After neutralisation with barium carbonate, the hydrolysate was separated on filter sheets (Whatman 3MM), with solvent B, to give fractions *a* (35 mg.; R_G 1·0) and *b* (25 mg.; R_G 0·80). These sugars were identified as 2 : 3 : 4 : 6-tetra- and 2 : 3 : 6-tri-*O*-methyl-D-mannose by conversion into their respective aniline derivatives, m. p. and mixed m. p. 141—143° and 120·5—123°. On reaction with phenylhydrazine 4-*O*-β-D-mannopyranosyl-D-mannose gave a phenylosazone, which crystallised in spherulitic aggregates of needles and had m. p. 203—206° (Found: *M*, 526, by absorption [9] at 396 mμ).

Fraction 2. The syrup (1·28 g.; eluted with water containing 6·5—8·0% of ethanol) contained three components, 4-*O*-β-D-mannopyranosyl-D-mannose, and disaccharides A and B, having $R_{mannose}$ 0·52, 0·62, and 0·38. Chromatographically pure samples of disaccharides were obtained by successive fractionations on columns of powdered cellulose and on filter sheets with solvent D.

Disaccharide A had $[\alpha]_D^{17}$ $+49°$ (*c* 0·6 in H_2O) and gave only mannose on hydrolysis. Periodate oxidation of the disaccharide in sodium hydrogen carbonate buffer [5] afforded formaldehyde, identified as the dimedon derivative, m. p. and mixed m. p. 187—190° (under

[8] Halsall, Hirst, and Jones, *J.*, 1947, 1399, 1427.
[9] Barry, McCormick, and Mitchell, *J.*, 1955, 222.

similar conditions no formaldehyde was formed from gentiobiose). A sample of the disaccharide (*ca.* 1 mg.) was dissolved in water (0·01 ml.) and glacial acetic acid (0·09 ml.), and lead tetra-acetate (5 mg.) in glacial acetic acid (0·4 ml.) was added. After 2 hr. excess of oxalic acid was added, and the solution was diluted with water and heated on the water-bath with Amberlite resin IR-120(H). Chromatographic examination of the hydrolysate showed erythrose but no arabinose (under similar conditions laminaribiose afforded arabinose). On reaction with phenylhydrazine the disaccharide gave a phenylosazone, m. p. 200—201° (Found: *M*, 600, by absorption [9] at 396 mμ).

Disaccharide B had $[\alpha]_D^{17}$ +5·5° (*c* 3·5 in H_2O) and gave mannose and glucose on hydrolysis. Hydrolysis of the derived aldonic acid gave only mannose. Periodate oxidation of the disaccharide in sodium hydrogen carbonate buffer [5] afforded formaldehyde, identified as the dimedon derivative, m. p. and mixed m. p. 187—190°. Lead tetra-acetate oxidation of the disaccharide followed by chromatographic examination of the hydrolysate gave erythrose but no arabinose. On reaction with phenylhydrazine the disaccharide gave a phenylosazone, m. p. 149—152° (Found: *M*, 542, by absorption at 396 mμ), which crystallised in leaflets and gave an *X*-ray powder photograph different from that of the phenylosazone from 4-*O*-β-D-mannopyranosyl-D-mannose.

Fraction 3. The sugar (5·6 g.; eluted with water containing 9·0—10·0% of ethanol) was crystallised from ethanol–water and had $R_{mannose}$ 0·22 in solvent A, m. p. (rapid heating) 134·5—135·5°, and $[\alpha]_D^{17}$ −15·7° ⟶ −20·2° (equil.) (*c* 1·29 in H_2O) (Found: C, 39·0; H, 7·1. Calc. for $C_{18}H_{32}O_{16},3H_2O$: C, 38·7; H, 6·9%). Whistler and Smith report m. p. 137—137·5° and $[\alpha]_D^{25}$ −24·7° ⟶ −23·3° for *O*-β-D-mannopyranosyl-(1 ⟶ 4)-*O*-β-D-mannopyranosyl-(1 ⟶ 4)-D-mannopyranose trihydrate, but the two samples gave identical *X*-ray powder photographs (comparison kindly undertaken by Professor R. L. Whistler). On slow heating on the Kofler hot-stage the sugar had m. p. 166·5—169·5° (presumably that of the anhydrous form); Dr. L. Hough [10] reports a similar observation. The sugar (300 mg.) was dissolved in water (6 ml.), and a solution of potassium borohydride (100 mg.) in water (2 ml.) was added. After 45 min. excess of borohydride was destroyed by the addition of dilute acetic acid, and the solution was taken to dryness. The residue was heated at 100° for 30 min. with acetic anhydride (12 ml.) and anhydrous sodium acetate (150 mg.). The solution was poured into ice-water; the precipitate after recrystallisation from ethanol–light petroleum (b. p. 80—100°) and from ethanol had m. p. 112·5—115° and $[\alpha]_D^{18}$ −24° (*c* 2·0 in $CHCl_3$) {Whistler and Smith [3] report m. p. 113·5—114° and $[\alpha]_D^{25}$ −21° (in $CHCl_3$) for mannotri-itol dodeca-acetate}.

Fraction 4. The syrup (1·84 g.; eluted with water containing 10·5—11·5% of ethanol) contained three components, mannotriose, and trisaccharides C and D, having $R_{mannose}$ 0·22, 0·29, and 0·15 respectively. Chromatographically pure samples of trisaccharides C and D were obtained after fractionation on cellulose with solvent C. Trisaccharide C crystallised from ethanol–water and had m. p. 224—225° and $[\alpha]_D^{18}$ +43° (5 min.) ⟶ +40° (1 hr., const.) (*c* 2·1 in H_2O) (Found: C, 42·9; H, 6·4. $C_{18}H_{32}O_{16}$ requires C, 42·8; H, 6·4%). Partial acid hydrolysis of the trisaccharide yielded mannose, mannobiose, and disaccharide A; partial acid hydrolysis of the derived glycitol (borohydride reduction) gave mannose and disaccharide A. When oxidised with periodate [11] the trisaccharide consumed 5·0 mol. after 8 hr. and 6·0 mol. after 24 hr.; *O*-β-D-mannopyranosyl-(1 ⟶ 4)-*O*-β-D-mannopyranosyl)-(1 ⟶ 4)-D-mannopyranose trihydrate consumed 5·0 ml. after 8 hr. and 5·95 mol. after 24 hr. Trisaccharide C (100 mg.) was dissolved in water (4 ml.) and a solution of potassium borohydride (40 mg.) in water (1 ml.) was added. The reaction mixture was kept at room temperature for 1 hr., excess of borohydride was destroyed by the addition of acetic acid, and inorganic salts were removed by passage through columns of Amberlite resins IR-100(H) and IR-4B(OH). The reduction product was methylated by successive additions of methyl sulphate and sodium hydroxide. The methylated glycitol (*ca.* 120 mg.), isolated from the mixture by chloroform extraction, was hydrolysed by 0·5N-sulphuric acid (10 ml.) for 3 hr. at 100°, and the hydrolysate was neutralised with barium carbonate, deionised, and concentrated. Separation of the reducing sugars in the hydrolysate on filter sheets with solvent C gave fractions *a* (42 mg.) and *b* (31 mg.). Fraction *a* was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-mannose by conversion into the aniline derivative, m. p. and mixed m. p. 142—144°. Fraction *b* was identified as 2 : 3 : 6-tri-*O*-methyl-D-mannose by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. 183—185°.

[10] Dr. L. Hough, personal communication.
[11] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.

Trisaccharide D had $[\alpha]_D^{17}$ $-7{\cdot}0°$ (c 4·1 in H_2O) and gave on hydrolysis mannose and glucose in the approximate ratio of 2 : 1. Partial acid hydrolysis of the trisaccharide yielded mannose, glucose, mannobiose, and disaccharide B; partial acid hydrolysis of the derived glycitol gave mannose and mannobiose.

Fraction 5. The sugar (2·1 g.; eluted with water containing 11·5—13·0% of ethanol) crystallised from ethanol–water, and after several recrystallisations had $R_{mannose}$ 0·11 in solvent A, m. p. 231·5—232°, and $[\alpha]_D^{20}$ $-31{\cdot}6°$ (5 min.) $\longrightarrow$ $-28{\cdot}7°$ (50 min., const.) (c 0·9 in H_2O). The sugar gave only mannose on hydrolysis and after partial acid hydrolysis chromatography showed the homologous series, mannose, mannobiose, mannotriose, and unchanged tetrasaccharide.

Fraction 6. The syrup (0·9 g.; eluted with water containing 13·0—14·5% of ethanol) contained mannotetraose together with samll quantities of a faster-moving component ($R_{mannose}$ 0·17). The minor component, separated on filter sheets with solvent A, gave only mannose on hydrolysis, and on partial acid hydrolysis mannose, mannobiose, mannotriose, disaccharide A, and trisaccharide C could be detected by chromatography.

Fraction 7. The sugar (0·9 g.; eluted with water containing 15—16% of ethanol) crystallised from ethanol–water and had $[\alpha]_D^{20}$ $-30{\cdot}2°$ (c 2·1 in H_2O); no m. p. (>280°) could be recorded. On partial acid hydrolysis, the homologous series of oligosaccharides, mannose, mannobiose, mannotriose, mannotetraose, and unchanged pentasaccharide were detected chromatographically.

Fraction 8. The syrup (0·8 g.; eluted with water containing 17—18% of ethanol) contained a complex mixture of oligosaccharides including a component having $R_{mannose}$ 0·08. A small quantity of this sugar was separated on filter sheets with solvent A and yielded on partial acid hydrolysis mannose, mannobiose, mannotriose, disaccharide A, and trisaccharide C.

Acetolysis of Mannan B and Fractionation of Derived Oligosaccharides.—Mannan B (13 g.) was added slowly to a mixture of acetic anhydride (80 ml.), glacial acetic acid (80 ml.), and concentrated sulphuric acid (8 ml.) at 0°. After 96 hr. at room temperature, the mixture was poured into ice-water (750 ml.), and sodium hydrogen carbonate was added slowly (to pH 3—4). The precipitated sugar acetates were filtered off and dissolved in chloroform, and the solution was combined with a chloroform extract (3 × 500 ml.) of the aqueous filtrate. The chloroform solution was washed with aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated to give a mixture of sugar acetates (14·6 g.). A portion of the sugar acetates (10·1 g.) was dissolved in dry methanol (150 ml.), and a solution of barium methoxide (1·35 g.) in methanol (15 ml.) was added. The mixture was shaken for 1 hr. and set aside at 0°. The mixture was worked up as described previously for mannan A and yielded a mixture of sugars (4·8 g.). The mixture of sugars dissolved in water (150 ml.) was added to a column of charcoal–Celite (300 g.; 1 : 1). Elution with water and ethanol–water (2 : 98) gave a mixture of monosaccharides (mannose, together with traces of xylose, glucose, and galactose; 0·42 g.) which was not examined further. Oligosaccharides were eluted with ethanol–water containing increasing proportions of ethanol. Elution with water containing 4—6% of ethanol gave 4-*O*-β-D-mannopyranosyl-D-mannopyranose (0·92 g.), m. p. and mixed m. p. 203—206°, which gave an *X*-ray powder photograph identical with that of an authentic sample. Elution with water containing 7—8% of ethanol yielded a syrup (84 mg.) shown by chromatography to contain three disaccharides (mannobiose, and disaccharides A and B) having $R_{mannose}$ 0·52, 0·62, and 0·38 in solvent A. Elution with water containing 9% of ethanol gave mannotriose trihydrate (600 mg.), which, after recrystallisation from ethanol–water, had m. p. and mixed m. p. 134·5—135·5°, and gave an *X*-ray powder identical with that of an authentic sample. Elution with water containing 10—11% of ethanol gave a mixture (166 mg.) of mannotriose, and trisaccharides C and D having $R_{mannose}$ 0·22, 0·29, and 0·15. Elution with water containing 12% of ethanol afforded mannotetraose, which, after recrystallisation from ethanol–water, had m. p. and mixed m. p. 231·5—232° and gave an *X*-ray powder photograph identical with that of an authentic sample.

Tests for Acid Reversion during Acetolysis.—Samples of mannose, mannobiose, and mannotriose (150—200 mg.) were each dissolved in 2 ml. of acetolysis mixture [acetic anhydride, acetic acid, and concentrated sulphuric acid, 10 : 10 : 1 (v/v)] at 0°. The solutions were kept at room temperature for 48 hr., filtered, and poured into ice-water, and sodium hydrogen carbonate solution was added. The sugar acetates, isolated by chloroform extraction, were deacetylated with barium methoxide in methanol at 0° for 18 hr., and the mixture was poured

into water. The resulting solution was deionised by passage through columns of Amberlite resins IR-120(H) and IR-4B(OH) and by electrodialysis with ion-exchange membranes,[12] and concentrated. Chromatographic examination of the products in solvent A showed only starting materials and hydrolysis products.

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[12] Anderson and Wylam, *Chem. and Ind.*, 1956, 191.