

**40. The Hemicelluloses of European Larch (*Larix decidua*).
Part II.¹ The Glucomannan Component.**

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Larch hemicellulose fractions rich in glucomannan were purified by fractional precipitations of the acetylated polysaccharides. Acetylated glucomannan contained residues of D-mannose (61%), D-glucose (31%), and D-galactose (7.5%). Hydrolysis of methylated glucomannan furnished tetra-, 2,3,6-tri-, 2,3-, and 2,6-di-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-galactose in the approximate molar proportions of 1:66:8:30:5. Partial acetolysis of acetylated glucomannan, followed by deacetylation, afforded a mixture of sugars including 4-*O*-β-D-mannopyranosyl-D-mannose, *O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannose, 4-*O*-β-D-mannopyranosyl-D-glucose, 4-*O*-β-D-glucopyranosyl-D-mannose, and cellobiose.

PREVIOUS studies on the polysaccharide components of European larch wood (*Larix decidua*) have been principally concerned with the xylan,¹ which can be extracted together with glucomannan on treatment of the holocellulose with dilute alkali, and with the water-soluble arabinogalactan (ε-galactan).² These structural investigations have now been extended to the glucomannan.

Larch hemicellulose fractions rich in glucomannan were obtained as described previously¹ by extraction of the wood holocellulose with 10% aqueous sodium hydroxide, and also by subsequent extraction with aqueous sodium hydroxide containing sodium borate.³ The glucomannan was isolated, virtually free from xylan, as its acetate after fractional precipitation of a mixture of acetylated polysaccharides. A sample of glucomannan, which was obtained on hydrolysis of the acetate, gave on hydrolysis mannose (61%), glucose (31%), and galactose (7.5%). The methylated polysaccharide was prepared by simultaneous deacetylation and methylation of the acetylated glucomannan. Hydrolysis of the methylated glucomannan afforded 2,3,4,6-tetra-*O*-methyl-D-mannose (identified by optical rotation, by chromatographic mobility, and by giving mannose no demethylation), 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl-D-glucose (all identified as crystalline derivatives), and a mixture of di-*O*-methyl-D-mannoses in the approximate molar proportions of 1:5:66:30:8. Chromatographic examination of the di-*O*-methylmannoses, and their products of demethylation and periodate oxidation, indicated the presence of 2,3- and 2,6-dimethyl ethers; it is possible that the fraction also contained a small proportion of a tri-*O*-methylgalactose. It follows that the polysaccharide preparation contains chains of 1,4-linked β-D-mannose and β-D-glucose residues in the proportion of *ca.* 2—2.5:1. The majority of D-galactose residues are present as non-reducing end groups and must arise from a galactomannan or galactoglucomannan. Mannose residues provide the only branching points in the polysaccharide, and although the nature of the linkages at the branching points has not been

¹ Part I, Aspinall and McKay, *J.*, 1958, 1059.

² Campbell, Hirst, and Jones, *J.*, 1948, 774; Jones, *J.*, 1953, 1672; Aspinall, Hirst, and Ramstad, *J.*, 1958, 593; Aspinall and Nicolson, *J.*, 1960, 2503.

³ Jones, Wise, and Jappe, *Tappi*, 1956, **39**, 139.

unambiguously established, it is clear that 1,6- and/or 1,3-linkages are involved and that the majority of side-chains are terminated by D-galactopyranose residues.

Partial acid hydrolysis of the glucomannan was effected by graded acetolysis of the polysaccharide followed by deacetylation of the products with barium methoxide. The resulting mixture of sugars was fractionated by chromatography on charcoal-Celite, followed where necessary by partition chromatography on cellulose. The following crystalline oligosaccharides were characterised: 4-O- β -D-glucopyranosyl-D-mannose, 4-O- β -mannopyranosyl-D-glucose, 4-O- β -D-glucopyranosyl-D-glucose (cellobiose), 4-O- β -D-mannopyranosyl-D-mannose, and O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose. Five of the six possible trisaccharides containing 1,4-linked β -D-mannose and D-glucose residues, although not obtained crystalline, were characterised by chromatographic examination of the partial hydrolysis products of the oligosaccharides and of the derived glycitols (from potassium borohydride reduction). These trisaccharides were: O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-glucose, O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose, O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-mannose, O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-glucose, and O- β -D-glucopyranosyl-(1 \rightarrow 4)-O- β -D-glucopyranosyl-(1 \rightarrow 4)-D-mannose. Four of these trisaccharides were isolated by Meier on partial acid hydrolysis of a glucomannan from Norwegian spruce⁴ and their ionophoretic mobilities were similar to those of our trisaccharides.

Small quantities were found of further oligosaccharides which probably do not represent fragments of the main polysaccharide. It is possible that 4-O- β -D-galactopyranosyl-D-galactose, arose from a small proportion of a 1,4-linked galactan of the type recently found in spruce compression wood.⁵ Since only traces of non-terminal galactose residues were found in the methylated polysaccharide, the methylated galactan may have been selectively removed during the methylation. Three oligosaccharides containing 1,4-linked α -D-mannose residues were tentatively identified. Although these sugars could have arisen from the presence of a small proportion of anomalous linkages in a predominantly β -linked polymer, it is more likely that these were artefacts from acid-catalysed anomerisation of β -D-mannopyranosyl linkages. It may be recalled that 4-O- α -D-mannopyranosyl-D-mannose and O- α -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose were formed in a similar manner from partial acid hydrolysis of ivory nut mannans,⁶ but that they could not be detected when the β -linked mannobiose and mannotriose were subjected to acetolysis followed by deacetylation. This control experiment has now been repeated with O- β -D-mannopyranosyl-(1 \rightarrow 4)-O- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose, and the products were separated by gradient elution from charcoal-Celite. Small amounts of the di- and tri-saccharide containing 1,4-linked α -D-mannopyranose units, and identified by chromatography and ionophoresis, were shown to be formed.

These results, therefore, indicate that European larch hemicellulose contains as a major component a glucomannan with main chains of 1,4-linked β -D-glucopyranose and β -D-mannopyranose residues in the proportion of 1:2.0—2.5. The isolation of 4-O- β -D-glucopyranosyl-D-mannose, 4-O- β -D-mannopyranosyl-D-glucose, and higher oligosaccharides with both sugar units provides clear evidence for a heteropolysaccharide. The D-galactopyranose residues in the polysaccharide are present largely as non-reducing end-groups and they are probably attached as side-chains to D-mannose residues in the main chains. It is not yet possible to assess the structural homogeneity of the larch polysaccharide, and the present results could be interpreted on the basis of *either* a single molecular species, a galactoglucomannan, or a mixture of a linear glucomannan and a

⁴ Meier, *Acta Chem. Scand.*, 1960, **14**, 749.

⁵ Bouveng and Meier, *Acta Chem. Scand.*, 1959, **13**, 1884.

⁶ Aspinall, Rashbrook, and Kessler, *J.*, 1958, 215.

galactomannan of the type found in the seeds of leguminous plants, *e.g.* carob gum⁷ or guar gum.⁸ It may be recalled that glucomannan fractions with and without galactose residues have been isolated from Sitka spruce wood.^{9,10} There is so far little detailed information concerning the regularity of distribution of glucose and mannose residues in the main chains of glucomannans, but the isolation of cellobiose and of trisaccharides containing the cellobiose unit as partial hydrolysis products shows that there are some contiguous glucose residues in the larch glucomannan. This polysaccharide is of the same general type as is commonly found in other coniferous woods, *e.g.* Western hemlock,¹¹ Western red cedar,¹² and various pines¹³⁻¹⁷ and spruces.^{4,9,10,18,19} Since this work was completed a structural study of a very similar glucomannan from tamarack (*Larix laricina*) has been reported.²⁰

EXPERIMENTAL

Paper chromatography was carried out on Whatman Nos. 1 and 3MM filter papers with the following solvent systems (v/v): (A) ethyl acetate-pyridine-water (10 : 4 : 3); (B) butan-2-one saturated with water containing 1% of ammonia; (C) butan-1-ol-ethanol-water (4 : 1 : 5, upper layer); (D) butan-1-ol-ethanol-water (1 : 1 : 1); (E) ethyl acetate-acetic acid-water (9 : 2 : 2). Methylated sugars were demethylated with hydrobromic acid. Optical rotations were observed at $18^\circ \pm 2^\circ$.

Extraction of Larch Hemicellulose and Preparation of Acetylated Glucomannan.—Larch hemicellulose fractions were extracted from the chlorite holocellulose as described in Part I¹ with the following modifications. Alkaline extractions were carried out by shaking the holocellulose with alkali in place of using a ball-mill; this procedure minimised the contamination of glucomannan fractions with degraded cellulose. Extracts with 10% aqueous sodium hydroxide were acidified; addition of acetone (200 ml. per 500 ml. of solution) afforded fraction A and further addition of acetone (300 ml.) gave fraction B. Samples of fractions A and B were hydrolysed by heating them with 95% formic acid at 100 for 6 hr. and then with *n*-sulphuric acid for 2 hr.; estimation of the sugars by Pridham's method²¹ showed galactose (6%, 4%), glucose (28%, 19%), mannose (54%, 42%), and xylose (12%, 35%).

Hemicellulose fractions were acetylated with acetic anhydride and pyridine in formamide by using Carson and Maclay's²² method. Chloroform-soluble acetylated hemicellulose A (29 g., from 50 g. of fraction A) had $[\alpha]_D -29^\circ$ (*c* 1.0 in CHCl_3) (Found: OAc, 42.5%). Precipitation of acetylated polysaccharide (29 g.) from chloroform (1800 ml.) by the addition of light petroleum (b. p. 60–80°; 2400 ml.) gave fraction A(i) (27 g.) (Found: OAc, 43.2%), $[\alpha]_D -23^\circ$ (*c* 1.0 in CHCl_3), which gave on hydrolysis mannose > glucose > galactose \gg xylose, and evaporation of the supernatant liquid gave fraction A(ii) (1 g.) (Found: OAc, 41.0%), $[\alpha]_D -39^\circ$ (*c* 1.0 in CHCl_3), which gave on hydrolysis xylose > mannose, glucose, and galactose. Fraction A(i) was used for acetolysis studies. Chloroform-soluble acetylated hemicellulose B (10 g., from 20 g. of fraction B) (Found: OAc, 42.7%), $[\alpha]_D -25^\circ$ (*c* 0.52 in CHCl_3), was dissolved in chloroform (1200 ml.); addition of light petroleum (b. p. 60–80°; 1600 ml.) gave fraction B(i) (7 g.). The supernatant liquid was evaporated and the residue was dissolved in chloroform; addition of ether gave fraction B(ii) (2 g.). Fraction B(i) (7 g.) was dissolved in chloroform

⁷ Smith, *J. Amer. Chem. Soc.*, 1948, **70**, 3249; Hirst and Jones, *J.*, 1948, 1278.

⁸ Whistler and Durso, *J. Amer. Chem. Soc.*, 1952, **74**, 5140, and earlier papers.

⁹ Aspinall, Laidlaw, and Rashbrook, *J.*, 1957, 4444.

¹⁰ Dutton and Hunt, *J. Amer. Chem. Soc.*, 1958, **80**, 5697.

¹¹ Hamilton, Kircher, and Thompson, *J. Amer. Chem. Soc.*, 1956, **78**, 2508; Hamilton and Kircher, *ibid.*, 1958, **80**, 4703.

¹² Hamilton and Partlow, *J. Amer. Chem. Soc.*, 1956, **78**, 4880.

¹³ Jones and Painter, *J.*, 1957, 669; 1959, 573.

¹⁴ Gyaw and Timell, *Canad. J. Chem.*, 1960, **38**, 1957.

¹⁵ Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 793; Perila and Bishop, *ibid.*, 1961, **39**, 815.

¹⁶ Croon, Lindberg, and Meier, *Acta Chem. Scand.*, 1959, **13**, 1299.

¹⁷ Hamilton, Partlow, and Thompson, *J. Amer. Chem. Soc.*, 1960, **82**, 451.

¹⁸ Lindberg and Meier, *Svensk Papperstidn.*, 1957, **60**, 785; Croon and Lindberg, *Acta Chem. Scand.*, 1958, **12**, 453.

¹⁹ Tyminski and Timell, *J. Amer. Chem. Soc.*, 1960, **82**, 2823.

²⁰ Kooiman and Adams, *Canad. J. Chem.*, 1961, **39**, 889.

²¹ Pridham, *Analyt. Chem.*, 1956, **28**, 1967.

²² Carson and Maclay, *J. Amer. Chem. Soc.*, 1946, **68**, 1015.

(400 ml.); addition of light petroleum (600 ml.) precipitated acetylated glucomannan B(iii) (5.7 g.), $[\alpha]_D -26^\circ$ (c 0.47 in CHCl_3) (Found: OAc, 42.1%), which was used for methylation studies. A sample of fraction B(iii) was deacetylated with sodium methoxide in methanol; hydrolysis of the product and estimation of the sugars by Pridham's method²¹ showed galactose (7.5%), glucose (32%), and mannose (61.5%). Fraction B(ii) (2 g.) was dissolved in chloroform (100 ml.); addition of light petroleum (150 ml.) gave a small precipitate which was rejected. The solution was concentrated, and the residue dissolved in chloroform and precipitated by the addition of ether. The precipitate had $[\alpha]_D -44^\circ$ (c 0.14 in CHCl_3) (Found: OAc, 40.8%), and gave on hydrolysis xylose and an aldobiouronic acid with only traces of mannose, glucose, and galactose.

Preparation of Methylated Larch Glucomannan.—Acetylated glucomannan [fraction B(iii), 5.5 g.] was methylated in acetone solution with methyl sulphate and 40% aqueous sodium hydroxide, giving a chloroform-soluble product (5.0 g.) (Found: OMe, 36.0. Calc. for methylated hexosan: OMe, 45.6%). Hydrolysis of a sample of this material followed by chromatography in solvent C showed tri-*O*-methylmannose and/or tri-*O*-methylglucose, together with much smaller amounts of tetra- and di-*O*-methylhexose, indicating a fairly complete degree of etherification of the polysaccharide despite the low observed methoxyl content. Further methylation of this material with methyl iodide and silver oxide gave a product (4.0 g.) with only slightly increased methoxyl content (Found: OMe, 37.0%). The methylated product was fractionated by solution in boiling mixtures of chloroform and light petroleum (b. p. 60–65°) which removed much non-carbohydrate material and gave methylated polysaccharide (2.0 g.) (soluble in chloroform–light petroleum (15 : 85)); this was reprecipitated from chloroform solution by addition of light petroleum to give methylated glucomannan (1.8 g.), $[\alpha]_D -8^\circ \pm 2^\circ$ (c 0.84 in CHCl_3) (Found: OMe, 42.2%).

Hydrolysis of Methylated Glucomannan and Separation of Methylated Sugars.—Methylated glucomannan (1.6 g.) was hydrolysed with boiling methanolic 3% hydrogen chloride (100 ml.) for 10.5 hr. and, after removal of methanol, with 0.5*N*-hydrochloric acid (140 ml.) at 100° for 14 hr. The hydrolysate was neutralised with silver carbonate; concentration of the filtrate gave a syrupy mixture of sugars (1.39 g.). The sugars were fractionated on cellulose (80 × 2 cm.), solvent B being used to give fractions 1–5. Fractions 1 (36 mg.) and 2 (192 mg.) apparently contained only tetra-*O*-methylhexoses, but hydrolysis of samples (2 mg.) indicated the presence of unhydrolysed methyl glycosides; fraction 3 (579 mg.) contained tetra-*O*-methylgalactose and tri-*O*-methylhexose; and fractions 4 (213 mg.) and 5 (76 mg.) contained tri- and di-*O*-methylhexoses, respectively. Accordingly fractions 1 and 2 were rehydrolysed with 0.5*N*-hydrochloric acid (20 ml.) at 100° for 5 hr. The hydrolysate (200 mg.) was combined with fraction 3 and separated on cellulose (70 × 3 cm.) with light petroleum (b. p. 100–120°)–butan-1-ol, saturated with water as eluant, to give fractions 1' (7.5 mg.), 2' (35 mg.), and 3' (522 mg.).

Fraction 1'. The sugar was chromatographically similar to 2,3,4,6-tetra-*O*-methyl-*D*-mannose and distinct from 2,3,4,6-tetra-*O*-methyl-*D*-glucose and -*D*-galactose in solvent C. Demethylation gave only mannose.

Fraction 2'. The sugar, $[\alpha]_D +116^\circ$ (c 0.45 in H_2O), was chromatographically indistinguishable from 2,3,4,6-tetra-*O*-methyl-*D*-galactose and was characterised as the aniline derivative, m. p. and mixed m. p. 188–190°.

Fraction 3'. The syrup was chromatographically similar to 2,3,6-tri-*O*-methyl-*D*-mannose and -*D*-glucose. The optical rotation in water $\{[\alpha]_D +17.5^\circ$ (c 1.0) $\}$ corresponded to that of a mixture of 2,3,6-tri-*O*-methyl-*D*-mannose (66%) and 2,3,6-tri-*O*-methyl-*D*-glucose (34%). The change in optical rotation in methanolic 3% hydrogen chloride $\{[\alpha]_D +35^\circ \longrightarrow -2^\circ$ (constant) (c 1.0) $\}$ indicated the presence in the mixture of 36% of 2,3,6-tri-*O*-methyl-*D*-glucose. A portion (257 mg.) of the syrup was kept in methanolic hydrogen chloride (25 ml.) at room temperature for 24 hr. (constant rotation), the solution was neutralised with silver carbonate, and the filtrate was concentrated to a syrup (200 mg.). The syrup was fractionated on filter sheets by using solvent C; the glycosidic component was hydrolysed with 0.1*N*-sulphuric acid (10 ml.) for 2 hr., and afforded a syrup (47 mg.) in which 2,3,6-tri-*O*-methyl-*D*-glucose was identified by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. 182–185°.

Fraction 4. The syrup, $[\alpha]_D +8.0^\circ$ (c 2.1 in H_2O), contained the same sugars as fraction 3', and the change in optical rotation in methanolic 3% hydrogen chloride $\{[\alpha]_D +26.6^\circ \longrightarrow +10^\circ$ (constant) (c 1.4) $\}$ indicated the presence in the mixture of 16% of 2,3,6-tri-*O*-methyl-*D*-glucose.

A portion (200 mg.) of the syrup was set aside in methanolic hydrogen chloride (10 ml.) at room temperature for 24 hr. (constant rotation), the solution was neutralised with silver carbonate, and the filtrate was concentrated to a syrup (180 mg.). After separation of the syrup on filter sheets by using solvent C, unchanged reducing sugar (103 mg.), $[\alpha]_D - 5^\circ$ (*c* 2.0 in H₂O), was identified as 2,3,6-tri-*O*-methyl-*D*-mannose by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. 187—188°.

Fraction 5. Chromatography of the syrup in solvent C showed a mixture of di-*O*-methylhexoses (R_G 0.54 and 0.60) and a trace of another sugar (R_G 0.65, possibly a tri-*O*-methylgalactose). Only mannose could be detected on demethylation. Separation of the mixture on a filter sheet in solvent C gave fractions 5(a) (17 mg.) and 5(b) (17 mg.). Fraction 5(a) had $[\alpha]_D + 13^\circ$ (*c* 0.67 in H₂O), and chromatography of the periodate-oxidation products²³ showed methoxymalondialdehyde (probably from 2,6-di-*O*-methylmannose) and three components indistinguishable from those derived from 2,3-di-*O*-methyl-*D*-mannose. Fraction 5(b) had $[\alpha]_D + 30^\circ$ (*c* 0.33 in H₂O) and chromatography of the periodate-oxidation products showed methoxymalondialdehyde and unchanged sugar of R_G 0.65.

Acetolysis of Acetylated Glucomannan and Fractionation of Derived Oligosaccharides.—Acetylated glucomannan [fraction A(i), 8.7 g.] was added slowly with stirring to a mixture of acetic anhydride (52 ml.), acetic acid (52 ml.), and concentrated sulphuric acid (5.2 ml.), and the mixture was kept at room temperature for 71 hr. The mixture was poured into water (300 ml.) and sodium hydrogen carbonate was added gradually (to pH 4—5). The precipitated sugar acetates were separated and dissolved in chloroform (150 ml.), and the aqueous solution was extracted with chloroform (4 × 150 ml.). The combined chloroform solutions were washed with sodium hydrogen carbonate solution, dried, and concentrated to a syrup (*ca.* 10 g.). Barium methoxide (1.8 g.) in methanol (9 ml.) was added to the syrupy acetates in chloroform (25 ml.) and methanol (50 ml.), and the mixture was kept at 0° for 20 hr. The mixture was poured into water (350 ml.), barium ions were precipitated by neutralisation with dilute sulphuric acid to pH 7, and the filtrate was concentrated to a syrup (5.0 g.). The mixture of sugars (5.0 g.) in water (130 ml.) was added to a column of charcoal-Celite (150 g.; 1 : 1). Elution with water yielded a mixture (1.14 g.) of monosaccharides (mannose, glucose, galactose, and a trace of xylose) which were not examined further. Elution with water containing increasing proportions of ethanol gave fifteen oligosaccharides, some of which were re-fractionated by chromatography on filter sheets by using solvents A, D, or E.

Oligosaccharide 1. The sugar (550 mg.; eluted with water containing 2.5% of ethanol), $R_{\text{glucose}} 0.50$ in solvent A, $M_G 0.66$, recrystallised from ethanol containing a trace of water, had m. p. and mixed m. p. 203—204°, $[\alpha]_D - 5.3^\circ$ ($\pm 0.7^\circ$) (equil.) (*c* 5.8 in H₂O), and gave an X-ray powder photograph identical with that given by 4-*O*-β-*D*-mannopyranosyl-*D*-mannose.

Oligosaccharide 2. The sugar (120 mg.; eluted with water containing 2.5—5.0% of ethanol), $R_{\text{glucose}} 0.40$ in solvent A, $M_G 0.34$, was recrystallised from ethanol-water and then had m. p. and mixed m. p. 199—201°, $[\alpha]_D + 33.7^\circ \longrightarrow + 19.7^\circ$ (equil.) (*c* 2.7 in H₂O), and gave an X-ray powder photograph identical with that given by 4-*O*-β-*D*-mannopyranosyl-*D*-glucose.

Oligosaccharide 3. The sugar (75 mg.; eluted with water containing 7.5% of ethanol), $R_{\text{glucose}} 0.85$ in solvent A, $M_G 0.60$, had m. p. (sealed tube) 128—130°, m. p. and mixed m. p. (Kofler hot-stage with sintering at 135—140°) 169—172°, $[\alpha]_D + 5.7^\circ \longrightarrow + 6.6^\circ$ (equil.) (*c* 1.2 in H₂O), and gave an X-ray powder photograph identical with that given by 4-*O*-β-*D*-glucopyranosyl-*D*-mannose monohydrate. The sugar was further characterised by conversion into 4-*O*-β-*D*-glucopyranosyl-α-*D*-mannose octa-acetate, m. p. and mixed m. p. 200—202°, and $[\alpha]_D + 34.9^\circ$ ($\pm 2.7^\circ$) (*c* 0.36 in CHCl₃), and X-ray powder photograph.

Oligosaccharide 4. The sugar (31 mg.; eluted with water containing 5.0—7.5% of ethanol), $R_{\text{glucose}} 0.53$ in solvent A, $M_G 0.25$, was recrystallised from ethanol-water and was then characterised as 4-*O*-β-*D*-glucopyranosyl-*D*-glucose (cellobiose) by m. p. and mixed m. p. 229—231°, $[\alpha]_D + 15.2^\circ \longrightarrow + 36.7^\circ$ (equil.) (*c* 1.2 in H₂O), and X-ray powder photograph.

Oligosaccharide 5. The sugar (8 mg.; eluted with water containing 2.5—5.0% of ethanol), $R_{\text{glucose}} 0.66$, $M_G 0.58$, $[\alpha]_D + 54^\circ$ ($\pm 5^\circ$) (*c* 0.4 in H₂O), gave only mannose on hydrolysis and was chromatographically and ionophoretically indistinguishable from 4-*O*-α-*D*-mannopyranosyl-*D*-mannose from ivory nut mannan.⁶

Oligosaccharide 6. The sugar (4 mg.; eluted with water containing 5.0% of ethanol), $R_{\text{glucose}} 0.40$, $M_G 0.49$, $[\alpha]_D + 59^\circ$ ($\pm 5^\circ$) (*c* 0.2 in H₂O), gave only galactose on hydrolysis, and

²³ Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

was chromatographically and ionophoretically indistinguishable from 4-*O*- β -D-galactopyranosyl-D-galactose but distinguishable from 3-*O*- β -D-galactopyranosyl-D-galactose. Oxidation of the sugar with lead tetra-acetate followed by hydrolysis²⁴ gave galactose and a tetraose (presumably threose) whereas similar treatment of 3-*O*- β -D-galactopyranosyl-D-galactose gave galactose and lyxose.

Oligosaccharide ^a	% EtOH in eluant	Yield (mg.)	R_{glucose} in solvent A	M_G ^b
8 $\beta\text{M}\rightarrow\beta\text{M}\rightarrow\text{G}$	5.0	25	0.10	0.40 (0.38)
9 $\alpha\text{M}\rightarrow\beta\text{M}\rightarrow\text{M}$	5.0	2	0.21 *	0.78 *
10 $\alpha\text{M}\rightarrow\beta\text{M}\rightarrow\text{G}$	7.5—10.0	10	0.15	0.49
11 $\beta\text{G}\rightarrow\beta\text{M}\rightarrow\text{M}$	10.0	11	0.24	0.49 (0.50)
12 $\beta\text{M}\rightarrow\beta\text{G}\rightarrow\text{G}$	10.0—12.5	44	0.14	0.31
13 $\beta\text{M}\rightarrow\beta\text{G}\rightarrow\text{M}$	12.5—17.5	100	0.21	0.57 (0.54)
14 $\beta\text{M}\rightarrow\beta\text{G}\rightarrow\beta\text{G}\rightarrow\text{M}$	20.0	22	0.07	0.40
15 $\beta\text{G}\rightarrow\beta\text{G}\rightarrow\text{M}$	17.5	20	0.29	0.49 (0.43)

Oligosaccharides obtained

	$[\alpha]_D$ in H_2O	Oligosaccharides obtained	
		on partial acid hydrolysis	on partial acid hydrolysis of derived glycolol
8	$-12^\circ \pm 3^\circ$	$\beta\text{M}\rightarrow\text{M}$, $\beta\text{M}\rightarrow\text{G}$	$\beta\text{M}\rightarrow\text{M}$
9		$\beta\text{M}\rightarrow\text{M}$, $\alpha\text{M}\rightarrow\text{M}$	$\alpha\text{M}\rightarrow\text{M}$
10		$\alpha\text{M}\rightarrow\text{M}$, $\beta\text{M}\rightarrow\text{G}$	$\alpha\text{M}\rightarrow\text{M}$
11		$\beta\text{G}\rightarrow\text{M}$, $\beta\text{M}\rightarrow\text{M}$	$\beta\text{G}\rightarrow\text{M}$
12	$+15.0^\circ \pm 0.8^\circ$	$\beta\text{M}\rightarrow\text{G}$, $\beta\text{G}\rightarrow\text{G}$	$\beta\text{M}\rightarrow\text{G}$
13	$-6.1^\circ \pm 0.2^\circ$	$\beta\text{M}\rightarrow\text{G}$, $\beta\text{G}\rightarrow\text{M}$	$\beta\text{M}\rightarrow\text{G}$
14	$-5.0^\circ \pm 0.5^\circ$	$\beta\text{G}\rightarrow\text{M}$, $\beta\text{M}\rightarrow\text{G}$, $\beta\text{M}\rightarrow\beta\text{G}\rightarrow\text{G}$	$\beta\text{M}\rightarrow\text{G}$, $\beta\text{G}\rightarrow\text{G}$ (tr.), $\beta\text{M}\rightarrow\beta\text{G}\rightarrow\text{G}$
15	$-5.5^\circ \pm 0.8^\circ$	$\beta\text{G}\rightarrow\text{M}$, $\beta\text{G}\rightarrow\text{G}$	$\beta\text{G}\rightarrow\text{G}$

^a M = D-mannopyranose, G = D-glucopyranose; in all cases 1,4-linkages are present. ^b Values in parentheses are M_G values quoted by Meier⁴ for trisaccharides formed on partial acid hydrolysis of a glucomannan from Norwegian spruce.

* Trisaccharide was compared with an authentic samples from ivory nut mannans.

Oligosaccharide 7. The sugar (40 mg.; eluted with water containing 5.0% of ethanol), R_{glucose} 0.17 in solvent A, M_G 0.55, was recrystallised from ethanol-water and was characterised as *O*- β -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose trihydrate by m. p. (sealed tube) 131—132.5°, m. p. and mixed m. p. 162—166° (slow heating on a Kofler hot-stage), $[\alpha]_D -13.5^\circ \rightarrow -20.3^\circ$ (equil.) (*c* 0.59 in H_2O), and X-ray powder photograph.

Oligosaccharides 8—15. The oligosaccharides formed on partial acid hydrolysis of the various syrupy higher oligosaccharides and of the derived glycolols (from reduction with potassium borohydride) were identified by chromatographic comparison with authentic samples. The details are given in the annexed Table.

Acetolysis of Mannotriose.—*O*- β -D-Mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannopyranose trihydrate (mannotriose) (1.0 g.) was treated with the acetolysis mixture [acetic anhydride, acetic acid, and concentrated sulphuric acid, 10 : 10 : 1 (v/v)] at 0° under the conditions used for the acetolysis of the glucomannan, and the resulting mixture of sugar acetates was deacetylated as described previously. The mixture (0.88 g.) was fractionated on charcoal-Celite by gradient elution with water containing 0.0—10.0% of ethanol, and the fractions were examined chromatographically in solvent A. The main fractions contained mannose, mannobiose, and mannotriose, but minor fractions contained sugars which were chromatographically and ionophoretically indistinguishable from 4-*O*- α -D-mannopyranosyl-D-mannose and *O*- α -D-mannopyranosyl-(1 \rightarrow 4)-*O*- β -D-mannopyranosyl-(1 \rightarrow 4)-D-mannose.

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