896. *The Glucomannans from Sitka Spruce* (Picea sitchensis).

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Hemicellulose fractions composed of D-mannose and D-glucose residues have been isolated from Sitka spruce holocellulose. A methylated glucan and a methylated glucomannan have been prepared therefrom, hydrolysis affording the corresponding 2 : 3 : 6-trimethyl ethers. It is concluded from these and other results that both glucan and glucomannan are linear polysaccharides composed of β-1 : 4-linked sugar residues.

Glucomannans have been isolated from a number of plant sources. The majority of these, for example, the glucomannans from iris seeds [1] and lily bulbs,[2] and the " Iles Mannan " from the tubers of some *Amorphophallus* species,[3,4] appear to function as reserve polysaccharides. All these materials contain essentially linear molecules composed of β-1 : 4-linked sugar residues. Of these polysaccharides, only in the case of " Iles Mannan " has evidence been brought forward to show that glucose and mannose are constituents of the same polysaccharide, rather than arising from a mixture of a glucan and a mannan. It is well known that coniferous woods contain cell-wall polysaccharides giving glucose and mannose on hydrolysis, but little is known concerning the mode or order of linkage of the constituent sugars. Anthis [5] has isolated from the partial hydrolysis of slash pine α-cellulose (containing 10% of mannan) two disaccharides 4-*O*-β-D-glucopyranosyl-α-D-mannose and a mannosylglucose which may be 4-*O*-β-D-mannopyranosyl-α-D-glucose; these disaccharides clearly arise from a glucomannan in the cell-wall structure. It is not

[1] Andrews, Hough, and J. K. N. Jones, *J.*, 1953, 1186.
[2] *Idem*, *J.*, 1956, 181.
[3] Rebers and Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 6097.
[4] Smith and Srivastava, *ibid.*, 1956, **78**, 1404.
[5] Anthis, *Tappi*, 1956, **39**, No. 6, 401.

clear, however, whether such a polysaccharide is a component of the hemicellulose fraction which has resisted extraction by alkali or whether the glucomannan participates in the highly ordered structure of the cellulose. Recently Jones and his collaborators [6] have fractionated the hemicelluloses of Loblolly pine and isolated a glucomannan which also contains a small proportion of D-galactose residues. Here again an essentially linear structure composed of 1 : 4-linked β-D-glucose and β-D-mannose units is indicated. Partial acid hydrolysis of this polysaccharide afforded the following oligosaccharides, 4-*O*-β-D-glucopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-glucose, and *O*-β-D-mannopyranosyl-(1 ⟶ 4)-*O*-β-D-mannopyranosyl-(1 ⟶ 4)-D-mannose.[7] These results were interpreted as indicating the presence of either a single glucomannan in which glucose and mannose residues are unevenly distributed, or a mixture of a glucomannan and one or more other mannans.

The present paper reports studies carried out on the glucomannans from Sitka spruce (*Picea sitchensis*). Partially delignified sawdust was extracted with cold aqueous alkali to give a complex mixture of polysaccharides yielding glucose, mannose, galactose, xylose, and arabinose on hydrolysis. The residual wood was completely delignified and the resulting holocellulose was extracted with cold 10% sodium hydroxide solution; the hemicellulose fraction after precipitation as the copper complex gave on hydrolysis glucose and mannose in approximately equal amounts. Methylation of the material resulted in the loss of the mannose-containing polysaccharide and a methylated glucan was isolated with an optical rotation indicating a β-linked polymer. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 4 : 6-tetra-*O*-methylglucose (identified by chromatographic mobility and by giving glucose on demethylation), 2 : 3 : 6-tri-*O*-methyl-D-glucose (identified as the crystalline sugar), and some di-*O*-methylhexose (probably arising from incomplete methylation of the polysaccharide). A molecular-weight determination by the isothermal-distillation method (by courtesy of Dr. C. T. Greenwood) gave a value of 8900 ± 500 (degree of polymerisation, 41—46) for the methylated polysaccharide. Although the quantity of non-reducing end-group isolated (1 in 80) was rather less than that required by a linear polymer, it is clear that this methylated polysaccharide is composed of unbranched chains of 1 : 4-linked β-D-glucopyranose residues and is indistinguishable on the present evidence from a methylated degraded cellulose.

The second sample of glucomannan, prepared in a similar manner, contained a higher proportion of mannose residues (mannose : glucose, 2·5 : 1). The consumption of periodate on oxidation was in excess of 1 mole per sugar unit; it is probable, however, that the over-consumption resulted from reactions other than α-glycol scission since the quantity of formic acid released was insufficient to indicate any large proportion of non-reducing end-groups or of 1 : 6-linked hexose units. The quantity of formic acid released during the oxidation was consistent with that from a linear hexosan of *ca.* 45 units. The polysaccharide was converted into a methylated glucomannan, whose optical rotation indicated a β-linked polymer. Hydrolysis of the methylated polysaccharide afforded a tetra-*O*-methylhexose (chromatographically indistinguishable from the 2 : 3 : 4 : 6-tetramethyl ethers of mannose and/or glucose but shown to be mainly the mannose derivative by optical rotation), a mixture of tri-*O*-methylhexoses, and some di-*O*-methylhexoses probably arising from incomplete methylation of the polysaccharide. Separation of the tri-*O*-methylhexoses was effected by selective methyl furanoside formation,[3] and the 2 : 3 : 6-trimethyl ethers of D-mannose and D-glucose were identified as their respective di-*p*-nitrobenzoates. The proportion of 2 : 3 : 6-tri-*O*-methyl-D-mannose to 2 : 3 : 6-tri-*O*-methyl-D-glucose was estimated as 3 : 1 from the optical rotation of the mixture of sugars in water and by the change of optical rotation (undergone solely by the glucose derivative) in methanolic hydrogen chloride. A molecular-weight determination by the isothermal-distillation method gave a value of 10,000 ± 500 (degree of polymerisation, 47—51) for

[6] Ball, J. K. N. Jones, Nicholson, and Painter, *Tappi*, 1956, **39**, No. 6, 438.
[7] J. K. N. Jones and Painter, *J.*, 1957, 669.

the methylated polysaccharide. This value taken together with the quantity of non-reducing end-group (1 in *ca.* 35) indicated that the polysaccharide has an essentially linear structure, although the possibility of a small proportion of molecules containing a single branch point cannot at present be excluded.

Partial acid hydrolysis of the glucomannan was effected by treating the polysaccharide with a mixture of acetic anhydride, acetic acid, and concentrated sulphuric acid at 0° and deacetylating the products with barium methoxide. It has been shown previously [5] that no reversion of monosaccharides occurs under these conditions. A preliminary chromatographic and ionophoretic examination of the products of partial acid hydrolysis showed the presence of glucose, mannose, cellobiose, mannobiose, and a mannosylglucose. The isolation of a mannosylglucose indicates that there is present in the spruce hemicellulose fraction a polysaccharide composed of both mannose and glucose residues.

These results indicate that the glucomannan fraction of Sitka spruce hemicellulose contains at least two essentially linear components, a β-1 : 4-linked glucan and a β-1 : 4-linked glucomannan. On the available evidence we cannot exclude the possibility also of a polysaccharide composed solely of mannose residues. The glucomannan component is clearly similar to the glucomannans previously examined from other plant sources.[1-4] It is increasingly evident from these results and from those of other workers [5-7] that in the coniferous woods there is no clear dividing line on grounds of solubility or of chemical structure between the ordered cellulosic framework, usually of high molecular weight, and the less highly ordered cell-wall polysaccharides or hemicelluloses, usually of lower molecular weight. In the present study it has been shown that polysaccharides composed solely of glucose units, and apparently differing from normal cellulose only in molecular size, may be extracted from the wood, together with glucomannan, by alkali. On the other hand, it is not yet clear whether the mannose units present in some cellulose preparations [5,8] are part of the true cellulose or arise from mannan or glucomannan occluded in the cellulose. It is noteworthy in this respect that the alkaline extraction of synthetic mixtures of ramie cellulose and ivory-nut mannan, prepared by denitration of a mixture of the nitrated polysaccharides, failed to remove all the mannan.[9] Further, evidence for the presence of mannose units in cellulose nitrates of high molecular weight [8] does not adequately prove the presence of mannose units in cellulose since fractional precipitation of mixtures of the nitrates of high-molecular-weight mannose-free ramie cellulose and relatively low-molecular-weight ivory-nut mannan afforded fractions of high molecular weight containing mannose units.[9]

Experimental

Paper 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) benzene–ethanol–water (170 : 50 : 15, upper layer). Paper ionophoresis was carried out in borate buffer at pH 10.[10]

Extraction of Sitka Spruce Glucomannan.—Air-dried sawdust (290 g.), which had been previously extracted under reflux with ethanol–water (85 : 15) and with benzene, was extracted successively with cold and hot water; small quantities of polysaccharide giving on hydrolysis galactose, glucose, and mannose, together with traces of arabinose and xylose, were isolated. Partial removal of lignin was effected by treatment of the residue with sodium chlorite solution acidified by the addition of acetic acid (procedure of Chanda, Hirst, Jones, and Percival [11]), yielding a spruce holocellulose (194 g., dry wt.) (Found: lignin, 5·5%). This material was extracted twice with cold 4% aqueous sodium hydroxide, and neutralisation of the extract with acetic acid followed by the addition of two volumes of ethanol afforded a polysaccharide fraction (9 g.) which gave on hydrolysis glucose, galactose, mannose, arabinose, and xylose.

[8] Timell, *Pulp and Paper Mag. Canada*, 1955, **56**, No. 7, 104.
[9] Snyder and Timell, *Svensk Papperstidn.*, 1955, **58**, 889.
[10] Consden and Stanier, *Nature*, 1952, **169**, 783.
[11] Chanda, Hirst, J. K. N. Jones, and Percival, *J.*, 1950, 1289.

Further extraction of the wood with cold 24% aqueous potassium hydroxide gave a similar complex mixture of hemicelluloses (11 g.). The residual wood was washed with water and delignified again to give a lignin-free residue (105 g., dry wt.). This material was extracted four times with 10% aqueous sodium hydroxide (2 l.) and the extracts were each treated with Fehling's solution (200 ml.). The gelatinous blue precipitates were combined and decomposed with acetic acid, and the resulting solid was washed with 80% acetic acid to remove copper salts and with acetone to remove acid and yielded a white powder. This material was twice precipitated from alkaline solution as the copper complex and regenerated to give glucomannan (sample A; 9 g.). Hydrolysis of the polysaccharide gave glucose and mannose in approximately equal quantities. Sample B, used in all the later experiments, was isolated in a similar manner except that the final reprecipitations *via* the copper complex were omitted. Sample B had $[\alpha]_D^{18}$ −33° (*c* 1·1 in 2N-NaOH) and chromatography of the hydrolysate showed the presence of mannose and glucose in the ratio of 2·5 : 1, together with a trace of xylose.

Methylation of Sample A.—Glucomannan (7·4 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide; neutralisation of the mixture followed by dialysis to remove inorganic salts gave partially methylated polysaccharide (5 g.) (OMe, 38·7%). After a further series of treatments with methyl sulphate and sodium hydroxide the methoxyl content of the methylated polysaccharide (3·5 g.) was raised to 41·6%. A further methylation with methyl iodide and silver oxide failed to raise the methoxyl content of the methylated polysaccharide (3·1 g.) beyond 42% {$[\alpha]_D^{20}$ −9·4° (*c* 3·5 in $CHCl_3$)} {cf. methylated cellulose $[\alpha]_D$ −4° (in $CHCl_3$) [12]}. The methylated glucomannan (1·7 g.) was hydrolysed by formic acid (40 ml.) at 100° for 5 hr.; after removal of formic acid the residual syrup was heated with N-hydrochloric acid (50 ml.) at 100° for 3 hr. After neutralisation with silver carbonate the hydrolysate was concentrated to a syrup (1·6 g.). The mixture of methylated sugars was fractionated on cellulose, with light petroleum (b. p. 100—120°)–butan-1-ol (6 : 4) saturated with water as eluant to give three fractions. Fraction 1 (18 mg.) travelled on the chromatogram at the same rate as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and gave glucose on demethylation. Fraction 2 (1·32 g.) crystallised and gave only glucose on demethylation. After two recrystallisations from ether–light petroleum (b. p. 60—80°) the sugar had m. p. and mixed m. p. (with 2 : 3 : 6-tri-*O*-methyl-D-glucose) 115—117°, $[\alpha]_D^{17}$ +100·5° (initial) ⟶ +70·6° (*c* 1·47 in H_2O), and $[\alpha]_D^{17}$ +58·5° ⟶ −36° (28 hr., constant) (*c* 1·49 in methanolic hydrogen chloride). Fraction 3 (93 mg.) contained a mixture of di-*O*-methylhexoses which were not examined further.

Periodate Oxidation of Glucomannan.—Samples of the glucomannan (*ca.* 30 mg.) were shaken in the dark with 0·3M-sodium metaperiodate solution (12 ml.), and determination of the periodate consumed gave the following results (moles consumed per $C_6H_{10}O_5$ residue) : 1·11 (48 hr.); 1·24 (117 hr.); 1·34 (261 hr.).

The glucomannan was oxidised with potassium metaperiodate solution, and the formic acid liberated was determined. The following results were obtained (expressed as the number of $C_6H_{10}O_5$ residues per mole of formic acid liberated); 18·0 (125 hr.); 15·1 (177 hr.); 13·5 (243 hr.); 12·4 (310 hr.). As the formic acid liberated did not reach a constant value, extrapolation to zero time gave a value corresponding to formic acid released from α-glycol scission, namely, 1 mole per 15 $C_6H_{10}O_5$ residues. The release of two mols. of formic acid from the reducing end-group and one mol. from the non-reducing end-group being assumed, this value corresponded to a chain length of 45 residues.

Methylation of Glucomannan.—The glucomannan (sample B) (2·5 g.) was converted into its thallium derivative which was heated with methyl iodide; [13] the product was treated three times in a similar manner and then methylated with silver oxide and methyl iodide, to give a methylated glucomannan (0·77 g.) (Found: OMe, 42·0%), whose methoxyl content could not be raised on further methylation {$[\alpha]_D^{18}$ −13° (*c* 2·48 in $CHCl_3$)}.

The methylated polysaccharide (0·65 g.) was heated with formic acid (20 ml.) in a sealed tube at 100° for 5 hr.; after removal of formic acid the residual syrup was heated with N-hydrochloric acid (25 ml.) at 100° for 3 hr., neutralised with silver carbonate, and concentrated to a syrup (0·54 g.). The mixture of methylated sugars was fractionated on cellulose (53 × 2·1 cm.), with butan-2-one saturated with water as eluant, to give four fractions.

Fraction 1 (15 mg.) had $[\alpha]_D^{18}$ +8° (*c* 1·8 in H_2O) and was chromatographically indistinguishable from the 2 : 3 : 4 : 6-tetramethyl ethers of D-mannose and D-glucose in solvents B and C.

[12] Haworth, Hirst, Owen, Peat, and Averill, *J.*, 1939, 1885.
[13] Fear and Menzies, *J.*, 1926, 937.

Fraction 2 (367 mg.) travelled on the chromatogram at the same rate as 2 : 3 : 6-tri-*O*-methyl-D-mannose and -D-glucose. The optical rotation in water {$[\alpha]_D$ +10·2° (*c* 3·5)} corresponded to that of a mixture of 2 : 3 : 6-tri-*O*-methyl-D-mannose (74%) and 2 : 3 : 6-tri-*O*-methyl-D-glucose (26%). The change in optical rotation in methanolic 1% hydrogen chloride {$[\alpha]_D^{17}$ +22° ⟶ −4° (*c* 0·49)} indicated the presence in the mixture of 25% of 2 : 3 : 6-tri-*O*-methyl-D-glucose. A portion (67 mg.) was dissolved in methanolic 1% hydrogen chloride and kept at room temperature for 20 hr. {$[\alpha]_D^{17}$ +22° ⟶ − 4° (constant)}. After neutralisation with silver carbonate and concentration the derived syrup was fractionated on cellulose (40 × 1·4 cm.) with butan-2-one saturated with water as eluant, to give fraction *a* (23 mg., non-reducing) and fraction *b* (41 mg., reducing). Fraction *a* was hydrolysed by heating with 0·1N-sulphuric acid at 100° for 2 hr., and the resulting reducing syrup (19 mg.) was identified as 2 : 3 : 6-tri-*O*-methyl-D-glucose by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. 187—189·5°. The 2 : 3 : 6-tri-*O*-methyl-D-mannose in fraction *b* was identified by conversion into the di-*p*-nitrobenzoate, m. p. and mixed m. p. (with sample m. p. 187—188°) 181·5—185° (a mixture of the di-*p*-nitrobenzoates of D-glucose and D-mannose trimethyl ethers had m. p. 167—173°). Fractions 3 (92 mg.; R_G 0·55 in solvent B) and 4 (20 mg.; R_G 0·37 in solvent B) contained mixtures of di- and mono-*O*-methyl-sugars which were not examined further.

Acetolysis of Glucomannan and Examination of Derived Oligosaccharides.—The glucomannan (1·0 g.) was dissolved in a mixture of acetic anhydride (12 ml.), acetic acid (12 ml.), and concentrated sulphuric acid (1·2 ml.) at 0° and set aside at room temperature for 120 hr. The mixture was poured into ice-water, neutralised with sodium hydrogen carbonate, and extracted with chloroform, and the extract was dried and concentrated to a syrup (1·0 g.). N-Methanolic barium methoxide (1 ml.) was added to a solution of the sugar acetates in methanol (25 ml.). The mixture was shaken at room temperature for 1 hr. and exactly neutralised with sulphuric acid, and the filtrate was concentrated to a syrup (360 mg.). Chromatography showed the presence of mannose, glucose, traces of galactose and xylose, two disaccharides ($R_{mannose}$ 0·52 and 0·38 in solvent A) and traces of other oligosaccharides. Samples of the two disaccharide components were isolated by fractionation on filter sheets, with solvent A. Component A gave a single spot on the chromatogram ($R_{mannose}$ 0·52) and gave mannose and glucose on hydrolysis, but paper ionophoresis showed two components to be present which had the same mobilities as cellobiose and 4-*O*-β-D-mannopyranosyl-D-mannose. Component B was chromatographically ($R_{mannose}$ 0·38) and ionophoretically identical with 4-*O*-β-D-mannopyranosyl-D-glucose and had $[\alpha]_D^{20}$ +9° (*c* 2·3 in H_2O). Hydrolysis afforded glucose and mannose whereas hydrolysis of the derived glycitol gave only mannose.

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