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The Constitution of a Glucomannan Associated with Wood Cellulose from Western Hemlock[1,2]

BY J. KELVIN HAMILTON AND HENRY W. KIRCHER

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A mixture of glucomannans was extracted with 18% sodium hydroxide from wood cellulose produced from western hemlock (*Tsuga heterophylla*) by the sulfite process. The mixture was acetylated and separated by fractional precipitation into fifteen fractions. Ten of these, corresponding to three-quarters of the mixture, had the same specific rotation, acetyl content, chemical composition, and differed only in their intrinsic viscosity. The ratio of D-glucose to D-mannose in this main fraction was 1:3. The molecular weight of one fraction was determined by osmometry. A single methylation of the purified glucomannan acetate yielded a product (45.3% OMe, $[\alpha]^{25}D$ −24.5°; *c* 3, $CHCl_3$) that was essentially homogeneous. Hydrolysis of the methylated polymer gave 2,3,6-tri-*O*-methyl-D-mannose together with smaller proportions of 2,3,6-tri-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose. About 1% of an unknown di-*O*-methyl- and 5% of an unknown tri-*O*-methylhexose also were separated. Graded hydrolysis of the glucomannan yielded 4-*O*-β-D-glucopyranosyl-D-mannose (glucosidomannose), 4-*O*-β-D-mannopyranosyl-D-mannose (mannobiose), 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose), 4-*O*-β-D-mannopyranosyl-D-glucose (mannosidoglucose), and a mannotriose. The significance of these findings and the relationship of this family of polysaccharides to the wood cellulose system in general is discussed.

The mannose-containing polymers that are present in conifers are an important group of carbohydrates from both a practical[3-5] and a theoretical[6-8] point of view. It is rapidly becoming apparent that true mannans (or polymers approaching a true mannan, such as ivory nut mannan) do not exist to any great extent in wood, and that the D-mannose is linked to other sugars, notably D-glucose and D-galactose, to form carbohydrate heteropolymers. The recent literature has dealt with the isolation of glucomannans and "galactomannans" from wood[9-12] and indications of their presence by partial hydrolysis of the wood[13] or of the polysaccharide[7] to yield oligosaccharides composed of D-glucose and D-mannose.

This paper is a continuation of our previous communication[14] and deals with the structure of a glucomannan that has been isolated from a wood cellulose prepared from western hemlock (*Tsuga heterophylla*) by the sulfite process.

A mixture of glucomannans and 4-*O*-methyl-D-glucuronoxylans was extracted from the wood cellulose[15] with 18% sodium hydroxide[16] and isolated by neutralization of the alkaline extract followed by dialysis, concentration of the salt-free aqueous solution, and precipitation of the polysaccharides with acetone. The 4-*O*-methyl-D-glucuronoxylans and a small quantity of the more soluble glucomannans were separated from the main portion of glucomannan polymers by a simple extraction with 0.1 *N* NaOH.[15]

The glucomannan ($[\alpha]_D^{25}$ −40.5°; *c* 4, 8% NaOH) could not be fractionated into significantly different portions with any aqueous solvent system such as water, ammoniacal copper solutions, borax, 0.1 *N* NaOH or 10% KOH. At best, the solvents removed the small quantity of xylose-containing polymer that remained with the glucomannan fraction and left a product having a specific rotation of −37.5° (*c* 4, 8% NaOH) and an intrinsic viscosity (I.V.) of 0.26 in 8% NaOH.

The fractional precipitation of the glucomannan acetate ($[\alpha]_D^{22}$ −23.9°, 9:1 tetrachloroethane–95% ethanol, subsequently called 9:1 solvent) from 9:1 solvent met with considerable success. Of the fifteen fractions that were obtained, the first four (25% of the total glucomannan acetate) were heterogeneous with respect to specific rotation, acetyl content, intrinsic viscosity and chemical composition. They were acetone insoluble and formed very turbid solutions in chlorinated hydrocarbon–alcohol solvent. They were not acetylated to full triacetates even after repeated acetylation at room temperature; however, more rigorous acetylation conditions would undoubtedly result in complete acetylation. Although these first four fractions had low acetyl contents and hence might be expected to have anomalous physical properties, hydrolysis of the fractions to their constituent sugars showed that they were a mixture of glucomannans in which the ratio of D-glucose to D-mannose was 2:1, 1:1 and 1:2, and suggested that the degree of acetylation was not solely responsible for the differences in their physical properties.[16a]

The next ten fractions constituted a series of glucomannan triacetates in which the ratio of D-glucose to D-mannose was 1:3. They had specific rotations very close to −30° (*c* 2.4, 9:1 solvent), acetyl contents corresponding to near triacetates, were acetone soluble, and differed from each other

(1) Contribution No. 31 from the Olympic Research Division, Rayonier Inc., Shelton, Wash.

(2) Presented at the 132nd Meeting of the American Chemical Society, New York City, September, 1957.

(3) K. E. Bradway, *Tappi*, **37**, 440 (1954).

(4) H. W. Steinmann and B. B. White, *ibid.*, **37**, 225 (1954).

(5) J. K. Watson and D. R. Henderson, *ibid.*, **40**, 686 (1957).

(6) D. H. Ball, J. K. N. Jones, W. H. Nicholson and T. J. Painter, *ibid.*, **39**, 438 (1956).

(7) J. K. N. Jones and T. J. Painter, *J. Chem. Soc.*, 669 (1957).

(8) J. Polglase, *Adv. Carbohydrate Chem.*, **10**, 283 (1956).

(9) J. K. N. Jones, L. E. Wise and J. P. Jappe, *Tappi*, **39**, 139 (1956).

(10) T. E. Timell and A. Tyminski, *ibid.*, **40**, 519 (1957).

(11) B. Immergut and B. Ranby, *Svensk Papperstidn.*, **60**, 573 (1957).

(12) G. A. Adams, *Tappi*, **40**, 721 (1957).

(13) A. Anthis, *ibid.*, **39**, 401 (1956).

(14) J. K. Hamilton, H. W. Kircher and N. S. Thompson, THIS JOURNAL, **78**, 2508 (1956).

(15) J. K. Hamilton and N. S. Thompson, *ibid.*, **79**, 6464 (1957).

(16) J. K. Hamilton and G. R. Quimby, *Tappi*, **40**, 781 (1957).

(16a) ADDED IN PROOF.—Part of this fraction recently has been separated into a pure glucan and glucomannan (glucose:mannose ratio of approximately 1:3) by the use of barium hydroxide as a selective precipitating agent in alkali. Meier[16b] has also observed the ability of barium hydroxide to precipitate mannose containing polymers.

(16b) H. Meier, *Acta Chem. Scand.*, **12**, 144 (1958).

only in their intrinsic viscosities (0.4 to 0.1, 8% NaOH). These results indicated that the latter portion of the separation was a simple chain length fractionation of a uniform heteropolymer composed of glucose and mannose in the ratio of 1:3 with properties that were distinctly different from the first one-quarter of the material that precipitated from solution.

A similar fractionation was accomplished very readily with acetone. The glucomannan triacetate dissolved in this solvent and left behind as an insoluble residue a mixture of the glucomannan acetates that were similar to those present in the first four fractions of the 9:1 solvent fractional precipitation.

The hemicelluloses and their derivatives, such as the acetylated glucomannans, are ideal materials for osmometry because their molecular weights (20–100,000) are in the range where the method is capable of giving reliable results. Work in this field has been delayed, however, by the recognized heterogeneity of the hemicelluloses that have been extracted from wood and the difficulties encountered in separation of the materials into physically and chemically homogeneous fractions. The early work by Miss Husemann on spruce mannan[17] and its benzoyl acetyl derivative showed the polysaccharide to have a degree of polymerization (D.P.) of 150–160.

The glucomannan triacetate that was employed for the osmometric studies was a sample from the 9:1 solvent fractional precipitation (see fraction 6, Table I). Its physical and chemical properties were representative of the major portion of the glucomannan that was extracted from the wood cellulose. The osmometrically determined value of 38,000 for the molecular weight of the glucomannan triacetate sample corresponds to a D.P. of 130, which may be too high because of the passage of low molecular weight material through the membrane during the osmometric determination.[18] The initial drop in the height of the liquid in the solution capillary that was observed may be explained in this manner. Similar difficulties have been reported recently by other workers studying low molecular weight polymers.[11]

In a previous communication of this series, the periodate oxidation data in conjunction with rotational evidence indicated that the glucomannan was predominantly a β-1→4-linked heteropolymer.[14] This has been confirmed by examination of the products that are formed by partial hydrolysis of the glucomannan. The hydrolyzate was separated into 4-*O*-β-D-glucopyranosyl-D-mannose, 4-*O*-β-D-mannopyranosyl-D-mannose, 4-*O*-β-D-glucopyranosyl-D-glucose, 4-*O*-β-D-mannopyranosyl-D-glucose and a mannotriose by a combination of charcoal–Celite and paper chromatography. The first three of these oligosaccharides crystallized, while the last two remained sirups. The distribution of D-glucose and D-mannose in the oligosaccharides suggested that the glucose and mannose anhydro units were randomly distributed along the glucomannan chain. The amount of glucosidomannose obtained, however, was four times as great as the amount of mannosidoglucose. A possible explanation may be the presence of an inordinately large fraction of the glucose in the glucomannan as the terminal non-reducing ends of polymer chains and/or that the bonds between glucose and mannose are more resistant to acid hydrolysis than the bonds between mannose and glucose.

The glucomannan was methylated from its acetate with powdered sodium hydroxide and methyl sulfate in tetrahydrofuran.[19] One treatment was sufficient to effect complete methylation (45.3% OMe) and the yield was quantitative. The product was fractionally precipitated and shown to be essentially homogeneous; it showed less degradation (higher intrinsic viscosity) than a similar product obtained *via* Haworth methylations.

Hydrolysis of the methylated glucomannan gave 2,3,6-tri-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,4,6-tetra-*O*-methyl-D-glucose in agreement with the structure of the glucomannan suggested by the previous experiments. These sugars were separated by paper chromatography using 2-butanone–water azeotrope as solvent. Two other unknown methylated sugars also were detected and isolated. One of these, which represented about 5% of the hydrolyzate, had a chromatographic mobility suggestive of a tri-*O*-methylhexose and had a rotation of +16° in methanol. This does not correspond to either 2,3,4-tri-*O*-methyl-D-mannose or 2,3,4-tri-*O*-methyl-D-glucose. The other unknown methylated sugar (about 1%) had a chromatographic mobility suggestive of a di-*O*-methylhexose and a rotation of +6° in water. Neither of the above two sugars showed any electrophoretic mobility using a borate buffer, thus indicating that position 2 was probably methylated. Independent experiments under similar conditions showed that 2,3,6-tri-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-xylose did not migrate, whereas 3,4-di-*O*-methyl-D-xylose did migrate.

The molar ratio of tetra- to tri-*O*-methyl sugars in the hydrolyzate with an almost complete absence of a di-*O*-methyl fraction would indicate a chain length considerably at variance with that indicated by periodate oxidation[14] and at an even greater variance than that obtained by osmometry. The explanation for this confliction of data can only be conjectured. It is possible that if the low recovery figures can be ignored (87–90%), either the tetra-*O*-methyl fraction of the hydrolyzate contained some methyl tri-*O*-methylhexoside or that some di-*O*-methylhexose was present in the tri-*O*-methylhexose fraction as a glycoside. Work on glucomannans from other woods indicates that these polymers are essentially strain chains, in contrast to the highly branched galactoglucomannans,[20] and therefore a negligible di-*O*-methylhexose fraction was not unexpected.

A possible structure for the glucomannan is I

(17) E. Husemann, *J. prakt. Chem.*, **155**, 12 (1940).

(18) A. J. Staverman, *Rec. trav. chim.*, **70**, 344 (1951).

(19) E. L. Falconer and G. A. Adams, *Can. J. Chem.*, **34**, 338 (1956).

(20) J. K. Hamilton, E. V. Partlow and N. S. Thompson, *Tappi*, in press.

$$G_{p1}-(_4M_{p1})_3-_4G_{p1}-(_4M_{p1})_4-(_4G_{p1})_2-(_4M_{p1})_2-$$
I

This structure explains nearly all of the data that have been obtained from periodate, methylation and graded hydrolysis studies. The straight chain nature of the polymer; the mannobiose, mannotriose, cellobiose, glucosidomannose and mannosidoglucose from the graded hydrolysis; the consumption of one mole of periodate per anhydro unit; the tetramethyl sugars (glucose in this instance); and the large quantity of 2,3,6-tri-*O*-methyl-D-mannose and tri-*O*-methyl-D-glucose from the methylation are all derivable from this postulated structure. Branch points other than 1→4 are not shown although there were indications of a small amount of a di-*O*-methylhexose and about 5% of an unidentified tri-*O*-methylhexose.

These glucomannans are common to most conifers, perhaps to all, and constitute the major proportion of the mannose-containing polymers in this type of wood. They are obtained readily from finely divided holo- or wood cellulose by simple extractions with concentrated sodium hydroxide solutions. The use of potassium hydroxide as an extraction agent has been shown to be ineffective for the removal of these dilute alkali-resistant polymers from wood cellulose. It has been found that even after isolation by sodium hydroxide extraction, the glucomannans are largely insoluble in potassium hydroxide. Another family of mannose-containing polymers that exist in conifers are the highly branched galactoglucomannans[12,20,21] which are readily soluble in dilute alkali and are not retained by the alkali-extracted residue. Galactoglucomannans, as well as the more predominant glucomannans, have been found in southern pine, western hemlock, western red cedar and other conifers.[20,22] In nearly all instances of the galactoglucomannans investigated in this Laboratory the galactose was found to be present as a single branch or the terminal unit of a longer branch as illustrated by the appearance of 2,3,4,6-tetra-*O*-methyl-D-galactose as the main galactose derivative observed in the hydrolyzate of the methylated polymers.

Thus it appears that in certain coniferous wood cellulose systems, glucomannans and galactoglucomannans exist as such, with the former predominating. The galactose is joined to the polymer by a labile linkage, as it may be selectively removed, together with some mannose by a dilute acid hydrolysis, to leave a type of glucomannan. It is known that the galactose residues are readily removed during sulfite pulping of wood, as sulfite pulps from conifers contain only trace amounts of galactose.[15] This behavior is analogous to the manner in which arabinose residues are removed from 4-*O*-methyl-D-glucuronoaraboxylan present in certain woods to give rise to a 4-*O*-methyl-D-glucuronoxylan in sulfite pulp, the selective removal of uronic acid residues to give rise to araboxylans in kraft pulp or the removal of both arabinose and uronic acid residues to give rise to xylans in prehydrolyzed kraft pulp.[20]

(21) G. G. S. Dutton and F. Smith, private communication.

(22) J. K. Hamilton and E. V. Partlow, THIS JOURNAL, **80**, Oct. (1958).

The amount of mannose-containing polymers remaining in wood celluloses has been correlated with the haze and anomalous viscosity of wood cellulose acetates in acetone.[3,4,5] A partial explanation for some of the haze and anomalous viscosity may lie in the acetone-insoluble fraction of the partially acetylated glucomannan that contains polysaccharides having a glucose-to-mannose ratio other than 1:3. The published experiments where mannose-containing polymers have been used to study the effect of "mannans" on the properties of wood cellulose or derivatives have been carried out using salep or ivory nut mannan. The results of such studies should not be considered as indicative of the effect that mannose-containing polymers from conifers would exhibit under similar conditions.

The purpose of the various hemicelluloses present in plant material in general, and in the wood cellulose system in particular, is highly thought-provoking. It has been suggested that certain polymers, such as the easily extractable branched uronic acid-containing polymers, may in some manner be joined to, or associated with, lignin. It is known that these polar polymers have a marked affinity for water and may thus act as a potential reservoir in arid weather. This type of polymer also appears to act as an intercellular cement. The role of the glucomannans is undoubtedly quite different. These polymers are very closely associated with the cellulose and constitute material that is termed "resistant" to extraction with alkali. They are predominantly straight chain, joined primarily by β-1→4-glycosidic bonds and, in this respect, are very similar to cellulose. The fact that they are of short chain length would indicate that they, by themselves, would not confer much strength to the skeletal stability of the tree. It is quite possible that these polymers exist primarily between the crystalline regions of the adjoining fibrils. This would explain the fact that caustic in the range of 8 to 10% must be employed in order to swell the cellulose and allow these compounds to dissolve in the strong alkali. Due to the large proportion of anhydromannose units, with their cisoidal 2,3-hydroxyl groups, the glucomannan may be held in close association by hydrogen bonds, add stability to the cellulose, and thus be involved in the skeletal system of the tree as a bonding or strengthening agent.

Experimental

Methods.—The quantitative sugar analyses were performed by paper chromatography[23] and later by a modification of an electrophoresis technique.[24] Glucose could be separated from mannose but not from xylose on a 0.05 *M* borax-impregnated paper. When boric acid was added to the borax solution to give an electrolyte containing 4% of the acid, the complicated metaborate (BO_2^-) ⇄ tetraborate ($B_4O_7^{=}$) ⇄ pentaborate ($B_5O_8^-$) equilibrium[25,26,27] was shifted

(23) M. Dubois, K. Gillis, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).

(24) D. R. Briggs, E. F. Garner, R. Montgomery and F. Smith, *ibid.*, **28**, 1333 (1956).

(25) A. Kesans, S. Vimba and E. Svarcs, *Latvijas PSR Zinatzu Acad. Vestis*, No. 7, 127 (1955); *C. A.*, **50**, 14420 (1956).

(26) E. Svarcs and A. Ievins, *ibid.*, No. 9, 135 (1956); *C. A.*, **51**, 10286 (1957).

(27) E. M. Shvarts and S. G. Vimba, *Zhur. Obshchei Khim.*, **27**, 23 (1957); *C. A.*, **51**, 13629 (1957).

enough to cause a difference between the strengths of the borate ion–glucose and borate ion–xylose complexes and enabled the two sugars to be separated.[28] The relative position of the mannose on the electrophoretogram was not affected, and the quantity of each sugar in a hydrolyzate was determined by a reflectance method similar to that of McCready and McComb[29] after the spots had been developed with a *p*-anisidine spray.

The glucomannan acetates were dissolved in 77% sulfuric acid prior to dilution and hydrolysis. The intrinsic viscosities of the glucomannan in oxygen-free 8% sodium hydroxide, its acetylated derivatives in 9:1 solvent (9:1 tetrachloroethane–95% ethanol) and methylated derivatives in chloroform were determined with a dilution viscometer.[30,31] At least four points on the reduced viscosity *versus* concentration curve were determined for each sample. The results are expressed in deciliters per gram (c = g./100 cc. solvent). The acetyl determinations were carried out by digestion of a small sample (30–40 mg.) with 77% sulfuric acid followed by distillation and titration of the acetic acid in the distillate.[32] When alcoholic alkali was used for the saponification of the glucomannan acetate samples instead of 77% sulfuric acid, the values that were obtained for the acetyl content were high and unreliable. Whatman Nos. 1 and 3 MM chromatographic papers, in conjunction with ethyl acetate–acetic acid–water 9:2:2 (solvent A) and 6:3:3 (solvent B), and 2-butanone–water azeotrope (solvent C) were used for the paper partition chromatographic separation of the sugars, oligosaccharides and methylated hexoses. Evaporations were carried out at reduced pressure and the melting points are corrected.

Isolation of the Glucomannan.—The extraction and separation of 4-*O*-methyl-D-glucuronoxylans and glucomannans from western hemlock wood cellulose was carried out as described previously.[14–16] The mixture of hemicelluloses (1.21 kg.) extracted from wood cellulose (18.7 kg.) showed $[\alpha]^{25}D$ −48.2° (c 3, 8% NaOH) and contained D-glucose, D-mannose, D-xylose and 4-*O*-methyl-D-glucuronic acid in the molar ratios 1:2:1.2:0.1. The respective composition of the wood before and after extraction was: α-cellulose, 89.4 and 96.2; β-cellulose, 2.3 and 2.2; γ-cellulose, 8.3 and 1.6; D-xylose, 3.4 and 0.6; D-mannose, 9.7 and 2.7.

Extraction of the mixture of hemicelluloses with 0.1 *N* NaOH dissolved the 4-*O*-methyl-D-glucuronoxylans (380 g.) and left behind a mixture of glucomannans (600 g., $[\alpha]^{25}D$ −40.5°; c 4, 8% NaOH; intrinsic viscosity 0.26 in cuprietylenediamine hydroxide and 0.24 in 8% NaOH). The glucomannans contained D-glucose, D-mannose and D-xylose in the molar ratios 1:2.5:0.1; ash (by ignition), 0.6%; moisture, 11.2%.

Acetylation of the Glucomannan.[33]—A suspension of the glucomannan (244 g.) in formamide (1060 ml.) was shaken for five hours. After adding pyridine (1530 ml.), acetic anhydride (1000 ml.) was added dropwise with stirring. The temperature of the reaction mixture was maintained at 20° with cooling until all of the anhydride had been added, after which the mixture was agitated for three days at room temperature. The glucomannan acetate was precipitated by pouring the reaction mixture into methanol (25 l.). The product was filtered, washed with methanol, ether and dried in air (yield 330 g.). The glucomannan acetate showed $[\alpha]^{25}D$ −24°; intrinsic viscosity, 0.23 (both in 9:1 solvent). It contained D-glucose, D-mannose and D-xylose in the molar ratio 1:2:0.1; Ac, 41.5%.

Fractionation of the Glucomannan Triacetate. A. From 9:1 Solvent.—A solution of 40.0 g. of the acetate in one liter of 9:1 solvent was prepared. The removal of about 0.5 g. of black, insoluble material by centrifugation and filtration through glass wool still left a very turbid, brownish-gray solution. Methanol (750 ml.) was added to this solution to precipitate the first fraction. Subsequent fractions were obtained by the addition of 250-ml. portions of methanol to the supernatant solutions from the previous precipitation. After the removal of the first three fractions, which were very dark gray, the solution had a clear yellow color and succeeding fractions were white or yellowish white. After the ninth fraction, 500 ml. of methanol was needed to precipitate fractions 10 and 11, and then the supernatant was concentrated in volume to 200 ml. because it had become too dilute with respect to the glucomannan acetate.

Twenty ml. of ethanol and 800 ml. of methanol, when added to this 200 ml., precipitated fraction 12; 300 additional ml. of methanol caused the separation of 13; and 750 ml. more precipitated 14. The fifteenth and last fraction was obtained by evaporation of the supernatant from 14 to a very small volume and pouring it into petroleum ether. The analysis of all of the fractions as well as the starting material is given in Table I.

TABLE I

FRACTIONAL PRECIPITATION OF THE GLUCOMANNAN TRIACETATE FROM 9:1 TETRACHLOROETHANE–ETHANOL WITH METHANOL

Sample		Wt., g.	Acetyl, %	9:1 Tetrachloroethane–95% ethanol: Specific rotation	9:1 Tetrachloroethane–95% ethanol: Intrinsic viscosity	Quantitative analysis, %: Glucose	Quantitative analysis, %: Mannose	Quantitative analysis, %: Xylose
Glucomannan triacetate		40	41.5	−23.9	0.23	1	2	0.1
Fract.	1	1.65	30.2	.....	0.27	2	1	0.07
	2	6.77	33.7	−18.1°	.22	1	1	.17
	3	1.72	36.7	−27.7	.23	1	2	.34
	4	1.70	29.2	−24.1	.20	1	2.3	.23
	5	2.85	40.5	−31.7	.30	1	3	Trace
	6	7.17	43.5	−30.5	.25	1	3.5	Trace
	7	2.25	43.1	−31.5	.21	1	3	0.26
	8	2.73	44.2	−30.1	.19	1	2.7	Trace
	9	2.90	44.2	−30.6	.17	1	3.3	None
	10	3.05	44.2	−30.1	.16	1	2.9	None
	11	1.15	44.3	−29.6	.14	1	3	None
	12	2.63	44.7	−29.7	.13	1	2.9	None
	13	0.95	43.4	−29.8	.13	1	3.1	None
	14	.07	43.2	−29.0	.11	1	3.2	None
	15	.62	39.5	−26.8	.10	1	2	None

B. From Acetone.—Fractions 6, 9 and 12 (500 mg.) dissolved in 25 ml. of acetone to form clear solutions. The unfractionated glucomannan triacetate dissolved partially in acetone, leaving 23% of the material as gray residue. The properties of the acetone-soluble and -insoluble fractions are given in Table II.

TABLE II

PROPERTIES OF ACETONE-SOLUBLE AND -INSOLUBLE GLUCOMANNAN ACETATES

Property	Acetone soluble	Acetone insoluble
$[\alpha]^{23}D$ (9:1 solvent)	−29.8°	−14.4°
I.V. (9:1 solvent)	0.21	0.26
Acetyl, %	44.0	35.4
Analysis (g:m:x)[a]	1:3.0:0.1	3:2:0.1
$[\alpha]^{23}D$ (8% NaOH)[b]	−39.3°	−32.6°
I.V. (8% NaOH)[b]	0.31	0.32

[a] Glucose:mannose:xylose. [b] Deacetylated polysaccharide.

Osmotic pressure measurements were carried out in a Hellfritz osmometer.[34,35,36] The membranes were cast from a

(28) J. K. Hamilton and H. W. Kircher, unpublished results.

(29) R. M. McCready and E. R. McComb, *Anal. Chem.*, **26**, 1645 (1954).

(30) E. B. Fitzgerald and R. M. Fuoss, *Ind. Eng. Chem.*, **42**, 1603 (1950).

(31) T. Alfrey, Jr., I. A. Goldberg and J. A. Price, *J. Colloid Sci.*, **5**, 251 (1950).

(32) E. P. Clark, *Ind. Eng. Chem., Anal. Ed.*, **8**, 487 (1936); **9**, 539 (1937).

(33) J. F. Carson and W. D. Maclay, THIS JOURNAL, **68**, 1015 (1946).

(34) H. Hellfritz, *Makromol. Chem.*, **7**, 184 (1951).

(35) H. Hellfritz and H. Kramer, *Kunststoffe*, **46**, 450 (1956).

(36) Hellma G.m.b.H. Glastechnische Werkstatten, Mulheim, Baden, Germany.

viscose solution made from cotton linters which contained no additives or plasticizers and were solvent exchanged (in the osmometer) from 50% ethanol through 95% ethanol and 50–50 95% ethanol–tetrachloroethane to 9:1 tetrachloroethane–95% ethanol. The 95% ethanol was used in preference to absolute ethanol because a mixed solvent that contains a small amount of water is recommended for cellulose derivatives to compensate for errors due to ions and residual moisture.[37]

A solution was prepared from 0.457 g. of moisture-free fraction 6 (Table I) and 44.61 g. of 9:1 solvent. Portions of this solution were diluted to yield three other solutions of 0.75, 0.5 and 0.25 of the original concentration.

The equilibrated osmometer was filled with the most dilute solution for a few minutes, emptied and refilled. The assembled instrument was placed in fresh solvent, and the height difference between the solvent and solution capillaries recorded as a function of time. Two consecutive determinations were run on each solution progressing from the most dilute to the most concentrated. These values extrapolated to zero time are shown in Fig. 1.

The equation M.W. = $25{,}300/(h/w)_0$[37] was used to calculate a molecular weight of 38,000 or a degree of polymerization of 130 for this fraction of the glucomannan triacetate. The latter value, together with the intrinsic viscosities of the glucomannan fraction in several solvents gave the following values for the Staudinger constant in the equation relating $[\eta]$ to D.P. ($[\eta] = k_m \times DP$): *glucomannan triacetate in 9:1 solvent*, intrinsic viscosity 0.25, k_m 1.9 × 10^{-3}; *glucomannan triacetate in acetone*, intrinsic viscosity 0.21, k_m 1.6 × 10^{-3}; *glucomannan in 8% sodium hydroxide*, intrinsic viscosity 0.39, k_m 3 × 10^{-3} (intrinsic viscosity constants expressed in g./100 ml.).

Graded Hydrolysis.—Preliminary experiments indicated that hydrolysis of the glucomannan by 0.2 *N* sulfuric acid (100°, 2 hr.), by solution in 72% phosphoric acid followed by dilution to a 1 *M* phosphoric acid solution and heating (100°, 1 hr.), and by acetolysis[38] all served to degrade the polysaccharide to the same oligosaccharides. Since simple dialysis[39] failed to separate the mono-, di- and trisaccharides, chromatography on charcoal–Celite[40] was employed. When the acetone-soluble and -insoluble fractions of the glucomannan acetate were deacetylated and subjected to a graded hydrolysis, no significant difference between the number and type of oligosaccharides produced from the two fractions was apparent.

A suspension of the glucomannan (130 g.) in water (1 l.) was stirred overnight, treated with 0.3 *N* sulfuric acid (2 l.) and heated for two hours at 95–102°. The mixture was cooled, filtered and the residue hydrolyzed twice more as before. The three hydrolyzates were separately neutralized ($BaCO_3$), deionized with Amberlite IR-140 (H+) and evaporated to a sirup; 84 g. unhydrolyzed glucomannan was recovered.

Paper chromatography using solvents A and B showed that the first hydrolyzate (32 g.) contained D-glucose, D-mannose, minor proportions of D-xylose and xylobiose (arising from a xylan impurity in the glucomannan) and a series of hexose oligosaccharides. The second (6.3 g.) and the third hydrolyzate (3.5 g.) contained D-glucose, D-mannose, traces of D-xylose and xylobiose; and the same series of hexose oligosaccharides found in the first hydrolyzate. The three hydrolyzates were combined, extracted with methanol (500 ml.) and the solution evaporated to a sirup (44 g.).

Charcoal Chromatography of the Graded Hydrolyzate.—A charcoal column was prepared on a large, sintered glass funnel. Darco G-60 and Celite were mixed (equal weights) and slurried in water. The mixture was poured as a slurry onto the funnel after the sintered glass had been covered with a wet piece of cellulose. The column itself was 15.5 cm. in diameter and 12.5 cm. deep. The methanol-soluble mono- and oligosaccharides (44 g.) were dissolved in 300 ml. of water, poured onto the column and allowed to soak into the charcoal. Then 48 liters of water were passed through the column to remove monosaccharides, followed by 18 l. each of 5, 7.5, 10 and 20% ethanol. Nine liters of eluent could be passed through the column every 24 hours. The four ethanol eluates were evaporated to dryness, extracted with methanol to leave behind a small amount (0.1–0.2 g.) of insoluble matter and examined chromatographically on solvents A and B. Their weights and contents are given in Table III.

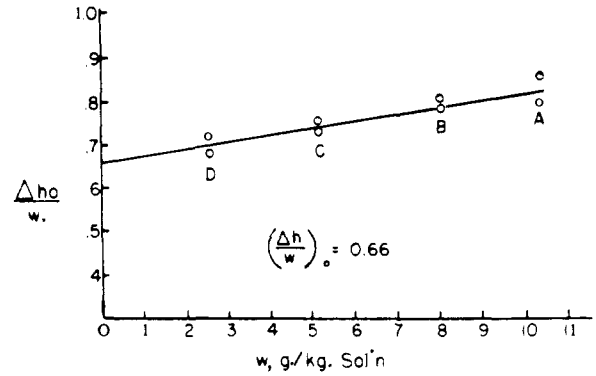

Fig. 1.—Osmotic pressure determination of glucomannan acetate: mol.wt. = R.T./$(\Delta h/w)_0$ = 25,300/0.66 = 38,000, D.P. = 38,000/288 = 130, $[\eta] = K$ (D.P.), K = 19 × 10^{-4}.

TABLE III

OLIGOSACCHARIDES PRODUCED BY GRADED HYDROLYSIS OF THE GLUCOMANNAN: RESULTS FROM CHARCOAL CHROMATOGRAPHY

Oligosaccharide		R_c[a]	Eluate; weight, g. 5% EtOH; 4.05 g.	7.5% EtOH; 3.44 g.	10% EtOH; 2.71 g.	20 EtOH; 4.08 g.
	1	1.92	+	+	+	
	2	1.45		++++	+++	+
Disaccharides	3	1.15	++++		+	+
	4	1.00		++		
	5	0.80	++			
	6	0.33			++	+++
Trisaccharides	7	.29	+	+++		+++
	8	.19		+		
	9	0.08			+	
Tetrasaccharides	10	0.05			+++	+++

[a] R_c is R cellobiose on solvent A (9:2:2 EtOAc:HOAc:H_2O).

Identification of the Oligosaccharides.—The oligosaccharides in the four ethanol eluates were further separated by chromatography on large sheets of Whatman 3MM paper with solvents A (4–10 days) and B (1.5–2 days). Oligosaccharide 1 appeared to be xylobiose (dark brown color with *p*-anisidine trichloroacetate spray and same R_f as authentic xylobiose on two solvents).

(a) **4-*O*-β-D-Glucopyranosyl-D-mannose (Glucosidomannose, Oligosaccharide 2).**—The material was obtained as a sirup (1.96 g.) that contained a very small amount of mannobiose as an impurity. It has R_c of 1.50 (solvent A) and 1.30 (solvent B), and yielded equivalent quantities of glucose and mannose upon hydrolysis. When a sodium borohydride reduction preceded hydrolysis, glucose was the only reducing sugar observed. After several days the sirup disaccharide crystallized spontaneously. When separated from sirup on a porous tile and recrystallized from aqueous ethanol, the 4-*O*-β-D-glucopyranosyl-D-mannose monohydrate had m.p. 133° dec., $[\alpha]^{25}D$ +5.5° equilibrium value in water (*c* 4); lit. m.p. 137°,[41] 134–139°,[38] 135–138°[7]; $[\alpha]^{23}D$ +14.5° → 5.9°,[41] +4.7°,[38] +6°[7] (water).

(b) **4-*O*-β-D-Mannopyranosyl-D-mannose (Mannobiose, Oligosaccharide 3).**—The disaccharide was obtained as a sirup (2.28 g.) that was chromatographically pure, R_c 1.20 (solvent A) and 1.11 (solvent B), and yielded only mannose upon acid hydrolysis. The sirup would not crystallize until it had been placed on another charcoal–Celite column (4.5 × 33 cm.) and eluted with 2 and 5% ethanol. Seed

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crystals were detected in one of the evaporated eluates, and all of the other eluates were nucleated. The crude disaccharide melted at 203–206° after recrystallization from cold methanol–water had m.p. 208–209°, $[\alpha]^{25}D$ −8.13° → −7.42° equilibrium value in water (*c* 3.5); lit. m.p. 193.5–194°,[42] 204°[7]; $[\alpha]^{23}D$ −7.7° → −2.2°,[42] −5° → −8°[7] (H_2O).

(c) **4-*O*-β-D-Glucopyranosyl-D-glucose (Cellobiose, Oligosaccharide 4).**—This disaccharide was a component of a crude sirup and was recognized by its chromatographic mobility on solvents A and B (R_c 1.00). The cellobiose crystallized spontaneously and was recrystallized twice with aqueous cold methanol and had m.p. 233–234°, mixed m.p. 233–234°, $[\alpha]^{25}D$ +26.7° → +35.1° equilibrium value in water (*c* 2.7); lit.[43] m.p. 225°, $[\alpha]D$ +14.2 → +34.6°; yield about 200 mg.

(d) **4-*O*-β-D-Mannopyranosyl-D-glucose (Mannosidoglucose, Oligosaccharide 5).**—This disaccharide was isolated as a sirup (~0.5 g.) which showed R_c of 0.80 (solvent A) and 0.87 (solvent B) and yielded equivalent quantities of glucose and mannose upon hydrolysis. Sodium borohydride reduction prior to hydrolysis left mannose as the only reducing sugar, showing that the disaccharide was a mannosidoglucose. The material was rechromatographed on charcoal to yield a chromatographically pure, colorless sirup; however, repeated attempts to crystallize the material failed. The identification of the disaccharide as 4-*O*-β-D-mannopyranosyl-D-glucose, therefore, rests upon its hydrolytic products before and after reduction and $[\alpha]^{25}D$ +20.1° (*c* 2.4, H_2O); lit. m.p. 202–203°,[38] 203°[7]; $[\alpha]^{25}D$ +30° → +19°,[38] +20°,[7] +35° → +18°.[7]

(e) **Mannotriose, Oligosaccharide 7.**—This trisaccharide was obtained in a fairly pure state because it could be desorbed from the charcoal–Celite column with 5 and 7.5% ethanol; the remaining trisaccharides were not eluted with these strengths. The sirup weighed 0.49 g. and had R_c of 0.28 (solvent A) and 0.56 (solvent B). On solvent A, a much weaker spot at R_c 0.19 was evident. The material was purified with charcoal from aqueous ethanol, but could not be induced to crystallize. It had a specific rotation of −15° (*c* 5, H_2O) and yielded only mannose upon complete acid hydrolysis. When the trisaccharide was hydrolyzed partially with Amberlite IR-140(H+) (100°, 2 hr.), unhydrolyzed starting material and substances which appeared to be mannobiose and mannose were the only sugars observed on the chromatogram. On the basis of the chromatographic mobility of the trisaccharide, the hydrolytic products and optical rotation, the material appeared to be a mannotriose.

One-step Methylation of the Glucomannan.—A solution of the glucomannan triacetate (6 g. acetone-soluble fraction, Table II) in acetone was dialyzed (cellophane sack) against acetone to remove low molecular weight impurities. The acetone solution was evaporated and the residue dissolved in dry tetrahydrofuran (100 ml.) and methylated with methyl sulfate (84 ml.) and solid sodium hydroxide (70 g.), the reagents being added in sevenths during two days.[21] After adding the second portions of reagents, the mixture was refluxed to complete the deacetylation. Thereafter the reaction was conducted at room temperature. During the methylation, tetrahydrofuran (150 ml.) was added to maintain fluidity. The reaction was completed by refluxing for 1 hour. The methylation mixture was cooled, filtered, and the residue dissolved in water and extracted with chloroform. The combined filtrate and washings were evaporated and the residual methylated glucomannan dissolved in chloroform (600 ml.). The chloroform solution was washed with water and evaporated to dryness *in vacuo*. After purification by extraction with acetone, the methylated glucomannan was obtained as a light yellow, crusty solid in quantitative yield (4.38 g.).

After purification by extraction with acetone and pouring the solution into petroleum ether, the methylated glucomannan (yield 3.69 g. or 87% of theory) had in chloroform $[\alpha]D$ −24.5° (*c* 3), and an intrinsic viscosity of 0.26 (Found: OCH_3, 45.3).

Fractionation of the Methylated Glucomannan.—The methylated glucomannan (3.6 g.) was dissolved in acetone (80 ml.) and fractionally precipitated in the usual way by adding increasing amounts of petroleum ether to give four fractions: fraction 1, 1.90 g., $[\alpha]D$ −24.5° ($CHCl_3$), intrinsic viscosity 0.32 ($CHCl_3$); fraction 2, 0.57 g., $[\alpha]D$ −26.8° ($CHCl_3$), intrinsic viscosity 0.32 ($CHCl_3$), fraction 3, 0.37 g., $[\alpha]D$ −24.5° ($CHCl_3$), intrinsic viscosity 0.20 ($CHCl_3$); fraction 4, 0.11 g., $[\alpha]D$ −20.4° ($CHCl_3$), intrinsic viscosity 0.14 ($CHCl_3$).

Hydrolysis of the Methylated Glucomannan.—Portions (1.60 g.) of fractions 1 and 2 were combined and dissolved in 25 ml. of methanol, 3 ml. of concentrated sulfuric acid in 25 ml. of water was added, and the mixture was heated on a boiling water-bath. As the methanol evaporated, it was replaced by water, and soon a clear aqueous solution was obtained which was refluxed overnight. It was neutralized ($BaCO_3$), deionized (Amberlite IR-140(H+)) and concentrated to a sirup. Paper chromatographic examination of the sirup (Solvent C) revealed: (a) a faint spot for a di-*O*-methylhexose, R_g 0.25; (b) a large spot for 2,3,6-tri-*O*-methyl-D-mannose, R_g 0.60; (c) a small spot for 2,3,6-tri-*O*-methyl-D-glucose, R_g 0.71; (d) a small spot for a sugar having R_g 0.87; (e) a small spot for 2,3,4,6-tetra-*O*-methylhexose, R_g 1.00 (R_g is the mobility of the sugar on the chromatogram relative to 2,3,4,6-tetra-*O*-methyl-D-glucose).

The hydrolyzate was separated on 17 large sheets of Whatman 3MM paper with solvent C in 4.5 hours in a chromatocab. The areas corresponding to di-, tri- and tetramethyl sugars were excised and eluted to yield the following ratio of methylated products: di-*O*-methylhexose (0.013 g.), tri-*O*-methylhexoses (1.092 g.) and tetra-*O*-methylhexoses (0.121 g.) which would give a molar ratio of 1:126:10, respectively. The mechanical losses in cutting and eluting 17 large sheets in order to obtain the five fractions (A to E) in a high degree of purity were about 10%. This would give an over-all yield of approximately 87–90%.

Identification of the Methylated Hexoses.—The individual sugars were purified by further chromatography on large sheets of paper (solvent C). The separation of the three trimethyl sugars was facilitated by sewing Whatman No. 50 wicks onto the Whatman 3MM papers to retard the rate of solvent flow down the chromatograms from 4.5 hours to 24 hours.[44]

(a) **Di-*O*-methylhexose** (R_g 0.25, solvent C).—This component which showed $[\alpha]^{25}D$ +6° (water), +16° (methanol) and +22° (acetone), displayed no electrophoretic mobility on paper in a borate buffer thus indicating C_2 was methylated. The di-*O*-methyl sugar did not crystallize and the corresponding acid failed to give either crystalline lactone or a phenylhydrazide.

(b) **2,3,6-Tri-*O*-methyl-D-mannose** (R_g 0.60, solvent C).—This component, isolated as an almost colorless sirup, showed $[\alpha]^{23}D$ −8.6° in water (*c* 7). When treated with aniline, it gave 2,3,6-tri-*O*-methyl-D-mannose-N-phenylglycosylamine, m.p. 121–124°, after recrystallization from petroleum ether and from ethanol; lit. for the free sugar $[\alpha]D$ +6°[45] and −10°[46]; and for the aniline derivative, m.p. 128°,[45] 131°,[46] 133°.[47]

(c) **2,3,6-Tri-*O*-methyl-D-glucose** (R_g 0.71, solvent C).—After removal of solvent, this component crystallized spontaneously and had m.p. 120–121°, $[\alpha]^{23}D$ +64.3°, equilibrium value in water (*c* 1.7) after recrystallization from ether; lit.[48] m.p. 121–123°, $[\alpha]D$ +70° (water).

(d) **Tri-*O*-methylhexose** (R_g 0.87, solvent C).—This component which showed $[\alpha]^{25}D$ 16° methanol (*c* 1), constituted about 5% of the hydrolyzate from the methylated glucomannan. It was obtained as a sirup and displayed no electrophoretic mobility on paper in a borate buffer, thus indicating C_2 was methylated. The rather high R_g on solvent C and the mobility on a paper chromatogram with 10:1:1 petroleum ether–butanone–methanol definitely placed it in the tri-*O*-methylhexose class.

(e) **2,3,4,6-Tetra-*O*-methyl-D-mannose** (R_g 1.00, solvent C).—The 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-mannose could not be separated on paper with a large number of different solvent systems. The

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most promising results were obtained with the petroleum ether solvent (above), but they could not be duplicated readily because of the volatility of the solvent and the formation of double fronts.

The tetra-*O*-methyl sugar fraction had $[\alpha]^{25}D$ +33° (*c* 2, H_2O) which indicated a 1:1 ratio of the two tetra-*O*-methylhexoses. The mixture (0.25 g.) was dissolved in 3 ml. of ethanol and refluxed for 6 hours with 0.2 ml. of aniline. The odor of aniline was still apparent, so 2 microliters of concd. HCl was added and the mixture refluxed briefly and allowed to stand overnight. It was evaporated to a sirup and extracted with a few ml. of ether. Petroleum ether was added to the ether solution until it became turbid and an oil formed. Crystals formed upon slow evaporation of the decanted supernatant solution and were recrystallized from an ether–petroleum ether mixture; m.p. and mixed m.p. with an authentic specimen of the N-phenylglycosylamine of 2,3,4,6-tetra-*O*-methyl-D-mannose was 143–144° (lit.[49] m.p. 144–145°).

(49) W. N. Haworth, R. L. Heath and S. Peat, *J. Chem. Soc.*, 833 (1941).

(f) 2,3,4,6-Tetra-*O*-methyl-D-Glucose (R_g 1.00, solvent C).—The mother liquors from the above crystallization of the tetramethylmannose were evaporated and the solid residue recrystallized from petroleum ether. Only enough material was available for a single m.p. determination. The crystals melted at 134–136° which corresponded to the literature[50] value (137–138°) of the m.p. of the N-phenylglycosylamine of 2,3,4,6-tetra-*O*-methyl-D-glucose.

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SHELTON, WASH.

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DEPARTMENT, U. S. NAVAL POWDER FACTORY]

The Reaction of Sodium Borohydride with Glycosyl Nitrates as Compared to its Reaction with the Nitrate Esters of the Primary and Secondary Alcohol Groups of Sugars[1]

BY F. A. H. RICE AND M. INATOME

RECEIVED MARCH 17, 1958

It has been found that sodium borohydride will reduce 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl nitrate and 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl nitrate at room temperature to yield the corresponding 1,5-anhydro alcohol. The nitrate esters of the primary and secondary alcohol groups of a sugar were not reduced.

Numerous studies on the chemistry of the nitrate esters of the sugars indicate that the reactions that any particular nitrate ester will undergo depend upon the position of the ester in the sugar molecule, other chemical groups present and also perhaps the stereochemical configuration of the sugar. When methyl 4,6-*O*-ethylidene-β-D-glucopyranoside 2,3-dinitrate reacts with sodium iodide in acetone the 2-nitrate group is replaced by hydroxyl while the 3-nitrate group is not attacked.[2] Similarly, dimethylamine will replace the 3-nitrate group in 6-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose 3,5-dinitrate with a hydroxyl group but will not replace the 5-nitrate group.[3] Pyridine is also known to be selective in its reaction with nitrate groups. Only the 3- and 4-nitrate groups are replaced by hydroxyl groups when either galactitol or D-mannitol hexanitrate[4] is treated with pyridine. The action of acidic and alkaline conditions has also been investigated[5] and it would seem that although extensive degradation usually occurs, acidic or alkaline conditions are selective in their action on sugar nitrates. Reducing agents, such as zinc and acetic acid[6] and sodium amalgam,[7] have been used to reduce the nitrate group to the parent alcohol. Catalytic hydrogenation[8] is not considered to be selective in the reduction of nitrate esters. It is impossible to be certain of this, however, since the reported reductions have been carried out over extended periods of time and hence any preferential reaction with specific nitrate groups might not become apparent, particularly if selectivity is due to differences in the rates of reaction with specific nitrate groups.

Lithium aluminum hydride, although it readily reduces simple nitrate esters,[9] shows some indication of selectivity in the sugar series. Methyl 4,6-*O*-propylidene-α-D-glucopyranoside 2,3-dinitrate on reduction yielded 19% of the 3-nitrate but none of the 2-nitrate.[10] Similarly the reduction of methyl 4,6-*O*-ethylidene-β-D-glucopyranoside 2,3-dinitrate on treatment with lithium aluminum hydride yielded 3% of the 3-nitrate.[11]

Since sodium borohydride is in general more selective in its action than lithium aluminum hydride[12] it was suspected that sodium borohydride might show more selectivity in its reaction with nitrate esters of sugars. Knowledge of such selectivity in the reduction of nitrate groups could not only be applied to the synthesis of a particular sugar nitrate but

(1) Published with the permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the authors.

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