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Studies on Glucomannans from Norwegian Spruce

III *. Partial Hydrolysis

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An easily extractable polysaccharide fraction from Norwegian spruce, containing galactose, glucose and mannose residues, was subjected to partial hydrolysis. A number of oligosaccharides were isolated from the hydrolysate; among them were the four disaccharides and five of the eight trisaccharides that should be formed from a linear glucomannan with β-1,4-linkages and a random distribution of the hexose residues. In addition to these compounds, 6-O-α-D-galactopyranosyl-D-mannose (Ga $\xrightarrow[\alpha]{1\ 6}$ M) and a trisaccharide (Ga $\xrightarrow[\alpha]{1\ 6}$ M $\xrightarrow[\beta]{1\ 4}$ M) were also isolated, indicating that, in addition to the glucomannan, there was also present either a galactomannan of the type found in legume seeds or a galactoglucomannan.

Glucomannans containing glucose and mannose residues in proportions of between 1:3 and 1:4 have been isolated from several coniferous woods. Methylation studies have shown that these polysaccharides are essentially linear with β-1,4-linked hexose residues [1–7], though some indications of branching have been found [3,7,8]. In agreement with this type of structure partial hydrolyses of the glucomannans or of pulps enriched in them [1,5,6,9–12] have afforded 4-*O*-β-D-mannopyranosyl-D-mannose (M $\xrightarrow[\beta]{1\ 4}$ M), 4-*O*-β-D-glucopyranosyl-D-mannose (*G* $\xrightarrow[\beta]{1\ 4}$ M), 4-*O*-β-D-mannopyranosyl-D-glucose (M $\xrightarrow[\beta]{1\ 4}$ G), cellobiose (G $\xrightarrow[\beta]{1\ 4}$ G) and mannotriose (M $\xrightarrow[\beta]{1\ 4}$ M $\xrightarrow[\beta]{1\ 4}$ M).

Some authors [1,4,6,13,14] have obtained polysaccharide fractions which in addition to glucose and mannose residues also contained a high percentage of galactose residues, indicating the presence of a galactomannan or a galactoglucomannan. It was observed in this laboratory that quite high galactose contents in some glucomannan fractions could be reduced to a low value (1 %

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Table 1. Carbohydrate analyses.

	Galactose %	Glucose %	Mannose %	Arabinose %	Xylose %
Norwegian spruce, extracted with acetone	2.1	70.7	17.2	1.3	8.7
Holocellulose (yield 71.8 %, Klason-lignin 1.4 %)	0.6	77.3	12.8	2.2	7.2
Crude polysaccharide fraction *A*	18.3	15.5	51.1	4.4	10.7
Purified polysaccharide fraction *B*	15.0	10.1	74.2	traces	0.7

or lower) if the polysaccharide material was delignified and precipitated with Fehling's solution [7]. It therefore seems probable that pine and spruce contain galactose-free glucomannans but that linkages between lignin and the polysaccharides make it impossible to separate material that has not been completely delignified, into its components. The most easily extracted, mannose-containing polysaccharide fraction has, however, not been dealt with in previous studies at this laboratory. The present paper describes an investigation of this fraction from Norwegian spruce (*Picea abies* Karst.).

Delignification of spruce wood by the chlorite method dissolves a large part of non-cellulose polysaccharides. This fraction (*A*, Table 1) was recovered by dialysis and was shown to consist mainly of polysaccharides containing mannose residues. To remove contaminating araboxylans, arabogalactans and galactans, *A* was purified by precipitation first with barium hydroxide [15] and then with Fehling's solution, giving fraction *B*. Galactans of the type recently isolated from compression wood [16] are precipitated by barium hydroxide but with Fehling's solution give little or no precipitate. Araboxylans and arabogalactans are not precipitated by either barium hydroxide or Fehling's solution. The material precipitated by barium hydroxide but not precipitated by Fehling's solution therefore contained a large proportion of galactose residues with lesser amounts of xylose and arabinose residues. The material precipitated by both barium hydroxide and Fehling's solution (fraction *B*, Table 1) was not free from lignin, so that contamination with other polysaccharides, *e.g.* a galactan, cannot be excluded, but it should consist essentially only of mannose-containing polysaccharides. *B* differed from other spruce polysaccharides containing mannose residues that have been studied in this laboratory [3,17], not only in its high content of galactose but also in the low ratio of glucose to mannose (1:7.4). *B* on electrophoresis on glass fibre sheets in borate buffer gave a single spot with considerable trailing.

Fraction *B* was subjected to partial hydrolysis, the yield of lower polysaccharides being improved by separation and further hydrolysis of the higher oligosaccharides. The oligosaccharides were then fractionated by carbon column chromatography, by chromatography and electrophoresis on thick filter paper and by chromatography on cellulose and carbon columns. Fifteen oligosaccharides (Table 2) were obtained in a state of purity. As can be seen from Table 2, the R_F values of some of these substances are very close, and separa-

Table 2. Oligosaccharides from the partially hydrolysed, mannose-rich fraction *B*.

	Oligosaccharide[a]	Number of combined[b] fractions	Yield in mg[c]	R_{Gl}[d]	M_G[e]	m. p.	$[\alpha]_D$	Ref.
a	M→M	49–59 (60–86)	810 + (155)	0.65	0.66	204–206°	− 8°	5, 12
b	M→G	60–86	17	0.48	0.43	201–203°	+19°	10, 11, 28
c	Ga→M	60–86	14	0.59	0.66	198–202°		30
d	Ga→Ga	87–95 (60–86)	30	0.57	0.50	208–210°		16
e	G→G	102–109	60	0.66	0.27	235–242° (dec.)		
f	G→M	110–134 (135–146)	650	0.87	0.58	140° (monohydrate) 179–182° (water free)	+ 6°	5, 12, 27–29
g	M→M→M	87–95 (60–86, 96–101)	216 + (189)	0.33	0.53			21
h	Ga→M→M	96–101 (102–109)	16	0.32	0.58			
i	M→M→G	96–101 (102–109)	29	0.28	0.38			
j	G→M→M	135–146 (147–156)	68	0.45	0.50			16
k	Ga→Ga→Ga	147–156	11	0.38	0.39			
l	M→G→M	50 % EtOH (181–186)	77 + (21)	0.39	0.54			
m	G→G→M	50 % EtOH	86	0.53	0.43			
n	M→M→M→M	102–109 (110–134)	227	0.15	0.50	235–250° (dec.)	−28°	
o	G→M→M→M (?)	157–163	19	0.21	0.45			

[a] All linkages are β-1,4, except Ga → in *c* and *h*, which are α-1,6.
[b] Numbers of fractions combined from the first carbon column fractionation. Numbers in brackets indicate that a substance was present in more than one of the combined fractions.
[c] As the separation and purification of the compounds was often tedious and involved considerable losses, the yields are not representative of the amounts present in the hydrolysate. The yields given in brackets are those for the combined fractions given in brackets, in the cases where the substance was isolated from these.
[d] Paper chromatographic mobility relative to glucose in solvent ii.
[e] Paper electrophoretic mobility in 0.1 M borate buffer, pH 10.0, relative to glucose.

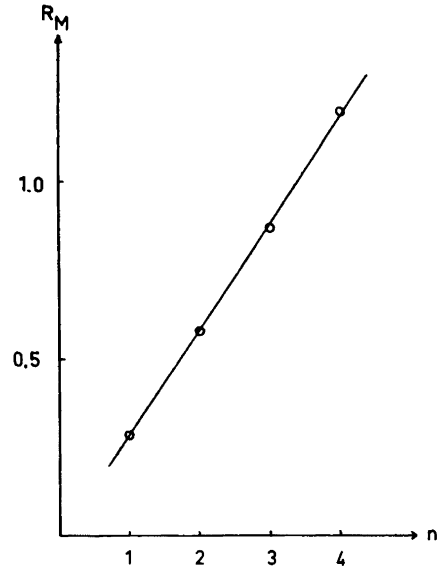

Fig. 1. Relation between R_M-values $[R_M = \log\left(\frac{1}{R_F} - 1\right)]$ and chain length.

tion could hardly have been effected without the aid of all of these methods. The results of the carbon column chromatography are rather surprising, some disaccharides being eluted after some of the trisaccharides and even after mannotetraose.

Definite structures could be assigned to all exept one (*o*, Table 2) of the fifteen oligosaccharides isolated. Total hydrolysis before and after reduction with sodium borohydride, and chromatography of the hydrolysate gave information on the component sugars and the reducing end group. Partial hydrolysis was carried out on the tri- and tetrasaccharides and the components obtained were identified by paper chromatography and paper electrophoresis. The degree of polymerisation of the tri- and tetrasaccharides was determined by the metod of Peat *et al.* [18].

The disaccharides (*a-f*, Table 2) were all known, crystalline compounds.

The mannobiose, mannotriose and mannotetraose which were obtained, belong to a homologous series, as is shown by the linear relationships between R_M and n [19] (Fig. 1) and between $[M]_D/n$ and $\frac{n-1}{n}$ [20] (Fig. 2). The mannotriose was obtained in an amorphous state in the present investigation but has been obtained crystalline by Whistler and Smith [21]; the value for $[M]_D$ in Fig. 2 is calculated from their value for the specific rotation. Fig. 2 gave the specific rotation of a β-1,4-linked mannan by extrapolation as $-48°$ (in water), in good agreement with the value $-46°$ (in N sodium hydroxide), found for ivory nut mannan A, which is known to contain principally these linkages [22].

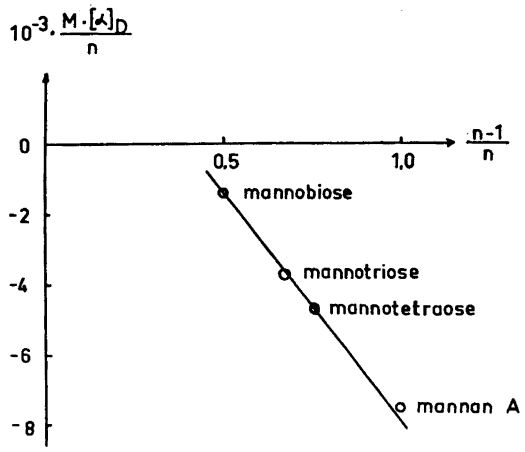

Fig. 2. Application of the Freudenberg-Blomquist relationship to mannose oligosaccharides (M = molecular weight, n = degree of polymerisation).

The galactobiose and galactotriose were obtained only in low yield and probably derive from a galactan of the type recently isolated from spruce compression wood [16].

The oligosaccharides containing both mannose and glucose residues obviously derive from a glucomannan of the ordinary type. The cellobiose may derive from contaminating cellulose, though this is less likely, especially as $G\xrightarrow[\beta]{1\quad 4}G\xrightarrow[\beta]{1\quad 4}M$ was also isolated. All of the disaccharides expected from a glucomannan and five of the eight theoretically possible trisaccharides were isolated. For statistical reasons, elements yielding cellotriose and to a lesser extent the trisaccharides with two glucose residues should be rare, but as glucosidic linkages are more resistant to acid hydrolysis than mannosidic, $G\xrightarrow[\beta]{1\quad 4}G\xrightarrow[\beta]{1\quad 4}M$ should be enriched compared to $M\xrightarrow[\beta]{1\quad 4}G\xrightarrow[\beta]{1\quad 4}G$ and $G\xrightarrow[\beta]{1\quad 4}M\xrightarrow[\beta]{1\quad 4}G$. Thus $G\xrightarrow[\beta]{1\quad 4}G\xrightarrow[\beta]{1\quad 4}M$ was the only trisaccharide with two glucose residues which was obtained in the present investigation. Oligosaccharides, derived from potential branching points in the glucomannan were looked for specially but were not observed. They would be present, however, only in small amounts and might easily have been overlooked.

Two oligosaccharides ($Ga\xrightarrow[\alpha]{1\quad 6}M$ and $Ga\xrightarrow[\alpha]{1\quad 6}M\xrightarrow[\beta]{1\quad 4}M$), containing an α-galactopyranosidic residue, linked to the 6-position in a mannose residue, were also obtained. Partical hydrolysis of the latter after reduction with sodium borohydride and identification of $Ga\xrightarrow[\alpha]{1\quad 6}M$ in the hydrolysate, eliminated the structure $Ga\xrightarrow[\alpha]{1\quad 6}M\xleftarrow[\beta]{4\quad 1}M$. The$Ga\xrightarrow[\alpha]{1\quad 6}M$ residue is known

as a structural element of the galactomannans from legume seeds, *e.g.* in locust bean gum and guaran[23]. These polysaccharides contain a linear chain of β-1,4-linked mannose residues, some of which are substituted in the 6-position by α-galactopyranosidic residues. It is therefore possible that the same type of polysaccharide occurs in spruce wood. These oligosaccharides could also be derived from a galactoglucomannan but the first possibility seems more attractive. If a more or less constant ratio of glucose to mannose of between 1:3.5 and 1:4 is assumed for the spruce glucomannans, then the carbohydrate composition of the present fraction (*B*) implies that it should contain about equal parts of glucomannan and galactomannan. The galactose to mannose ratio in the latter should be lower than 1:2.5, as there are indications that galactans are also present in fraction *B*. The isolation of the two polysaccharides in a state of purity should of course definitely prove this hypothesis, but the separation of a glucomannan and a galactomannan by present methods seems to be a most difficult task. The isolation of $G\xrightarrow[\beta]{1\quad 4}G$ and $G\xrightarrow[\beta]{1\quad 4}G\xrightarrow[\beta]{1\quad 4}M$ from the hydrolysate gives some support to the presence of a glucomannan (glucose:mannose, 1:4), since structural elements giving these oligosaccharides should be even more rare in a galactoglucomannan, in which the proportions of glucose to mannose are 1:7.4.

In methylation studies of mannose-rich polysaccharide fractions from Loblolly pine and Sitka spruce Ball *et al.*[1] and Dutton and Hunt [4] isolated 2,3,4,6-tetra-*O*-methyl-D-galactose and a di-*O*-methyl-D-mannose, believed to be the 2,3-dimethyl-ether. This is in good agreement with our results, which show the presence of galactose residues that are linked to 6-positions in mannose residues and are most probably terminal.

In a recent paper [24] Hamilton and Thompson have reported the isolation and a structural investigation of a polysaccharide, believed to be a galactoglucomannan, from southern pine.

EXPERIMENTAL

Melting points are corrected. Concentration of solutions was done under reduced pressure at a bath temperature of 40°.

Paper chromatography and paper electrophoresis. Whatman No. 1 and No. 3 MM papers were used. The chromatograms were run in the solvent systems (v/v):

i. ethyl acetate-acetic acid-water, 3:1:3 (upper layer)
ii. ethyl acetate-pyridine-water, 2:1:2 (upper layer)
iii. acetone-butan-1-ol-water, 7:2:1.

The method of Saeman *et al.*[25] was used for quantitative determination of monosaccharides. Paper electrophoreses were run in 0.1 M borate buffer of pH 10.

Isolation and fractionation of the polysaccharides. Norwegian spruce wood meal (1 000 g, 0.5—2.0 mm) was extracted with acetone and the residue was delignified by the chlorite method at 60° and pH 4.7. The product remaining after delignification was 71.8 % of the acetone-extracted wood. The combined chlorite solutions (30 l) were dialysed against tap water for five days and then concentrated to about 5 l. After acidification with acetic acid the hemicelluloses were precipitated with 20 l ethanol. The precipitate (*A*, Table 1) was washed with aqueous ethanol, ethanol and then ether and dried (yield 94 g, containing 16 % lignin, determined spectrophotometrically [26]). A sample of this material (70 g) was dissolved in water and saturated barium hydroxide solution was added until precipitation was complete. The precipitate was centrifuged, washed, dis-

solved in dilute acetic acid and the polysaccharides were reprecipitated with ethanol and washed. The product was dissolved in water, Fehling's solution was added, the precipitate formed was centrifuged, washed, dissolved in dilute acetic acid, and the polysaccharides (*B*, Table 1) were recovered by precipitation with ethanol (yield 25 g). The centrifugate, after addition of Fehling's solution, was acidified and dialysed, and the polysaccharides were precipitated with ethanol (yield 8 g). This fraction yielded, after hydrolysis, mainly galactose, with smaller amounts of arabinose, xylose and uronic acids.

Partial hydrolysis of B. A sample of *B* (15 g) dissolved in N sulphuric acid (1 000 ml) was heated on the steam bath for 30 min and then was cooled and neutralised with barium hydroxide. Barium sulphate was removed by centrifuging and the solution was shaken with activated carbon (400 g) and then filtered. The aqueous phase contained only monosaccharides and was discarded. The carbon was eluted first with 15 % and then with 50 % aqueous ethanol. The 15 % ethanol eluate contained monosaccharides and lower oligosaccharides. The second eluate contained higher oligosaccharides, which were subjected to further partial hydrolysis; the whole procedure was repeated twice. The 15 % ethanol eluates and the final 50 % ethanol eluate were combined, concentrated and added to the top of a carbon-Celite column (4 × 45 cm). Gradient elution with aqueous ethanol (6 l, 0—20 %) effected a partial separation of the components (Table 2). In addition to the substances listed in Table 2, some higher, unidentified oligosaccharides were observed in fraction 110 and later fractions. Similar fractions from the carbon column were combined giving eleven main fractions. A twelfth fraction was obtained by elution of the column with 50 % ethanol. Most of the main fractions were fractionated further by chromatography on cellulose columns or on thick filter paper. Sometimes a single separation was sufficient, but for some of the mixtures two successive fractionations in different solvent systems were necessary. For some mixture, *e. g.* substances *c* and *d*, electrophoresis on thick filter paper was also used for separation. The purification of *h* from contaminating *g*, was done by carbon column chromatography.

Identification of the oligosaccharides. The oligosaccharides were identified as described above.

Ga $\overset{1}{\underset{\alpha}{\longrightarrow}}\overset{6}{}$ M was compared with an authentic sample, kindly supplied by Professor Roy L. Whistler. The two samples were chromatographically and electrophoretically indistinguishable, gave an undepressed mixed m. p. and also gave identical X-ray powder diagrams. The lattice spacings in Å were: 6.57 (m), 6.33 (s), 5.22 (vs), 5.06 (m), 4.55 (m), 4.42 (vs), 4.28 (m), 4.18 (m), 4.05 (m-s), 3.69 (s), 3.52 (s).

Substance *f*, (G $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M) had a specific rotation +6° (in water). Similar values [5,27,28] and some higher values, +12° and +15° [11,29] have been reported for this substance.

Component *o* on hydrolysis yielded one part of glucose to three parts of mannose. After borohydride reduction and hydrolysis the proportions were one to two. A paper chromatographic and electrophoretic examination of a partial hydrolysate of *o* indicated the presence of G, M, G $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M, M $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M and G $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M. Even though M $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M was not detected, the structure G $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M $\overset{1}{\underset{\beta}{\longrightarrow}}\overset{4}{}$ M appears to be the most probable for this substance.

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