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Structure of galactoglucomannan from *Populus monilifera* H.

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A polysaccharide containing D-galactose, D-glucose and D-mannose in the mole ratio of 1:4·1:9·7 has been isolated from poplar (*Populus monilifera* H.) wood. Structural studies were performed by glycosyl linkage composition analysis, ^{13}C-NMR spectroscopy and partial hydrolysis. From these experiments a structure for the polysaccharide is proposed.

INTRODUCTION

Galactoglucomannans are the structural constituents of woody tissues of gymnosperms and angiosperms (Timell, 1965). The presence of this class of polysaccharides in different tissues of many other plants and in the seeds of various land-plants has been reviewed (Aspinall, 1959; Whistler & Richards, 1970; Dey, 1980).

The results of recent work have proved that these mannose-rich polysaccharides, together with fucogalactoxyloglucan and xylan, are also components of plant primary cell walls (Little *et al.*, 1980; Akiyama *et al.*, 1983; Thomas *et al.*, 1983). Biological activity of the derived xyloglucan fragments has been evidenced (Darvill *et al.*, 1985; McDougall & Fry, 1989; Aldington *et al.*, 1991). The possible effect of the fragments of other minor components, such as the galactoglucomannans of both the primary and the secondary cell walls of higher plants on biological activity, has not been reported.

Therefore, this paper deals with the isolation and structural characterization of a galactoglucomannan from the wood of poplar and derived oligosaccharides. The biological activity of these components will be the subject of a future investigation.

MATERIAL AND METHODS

General

All evaporations were conducted under diminished pressure at 40°C. Optical rotations (1 ml cell) were measured at 20 ± 1°C with a Perkin–Elmer Model 141 polarimeter. Free boundary electrophoresis of polysaccharide solutions (10 mg/ml) was performed in 0·05 M sodium tetraborate buffer (pH 9·2) using a Zeiss 35 apparatus run at 180 V and 6 mA for 30 min.

Gas-liquid chromatography (GLC) was carried out on a Hewlett-Packard Model 5711 A instrument with a column (305 × 0·3 cm) of 1% XE-60 on Gas Chrom Z (80–100 mesh) at 130–150°C (1°C/min) with an N_2 flow of 36 ml/min column (a); a column (200 × 0·3 cm) of SP-2340 on Chromosorb WAW-DMCS (80–100 mesh) at 180°C (4 min)–210°C (2°C/min), flow rate of N_2 30 ml/min (b); a column (400 × 0·4 cm) of 10% Carbowax 400 on Chromosorb WAW (80–100 mesh) at 45°C, with an N_2 flow rate of 17 ml/min column (c). Column (a) was used for quantitative analysis of sugar trifluoroacetates.

Gas-liquid chromatography – mass spectrometry (GLC–MS) was carried out with a JGC-20 K gas chromatograph fitted with column (b), and with helium (inlet pressure, 101·3 kPa) as the carrier gas. Mass spectra were obtained at 23 eV and an emission current of 300 μA, using a JMS-D 100 (JEOL) spectrometer. The inlet temperature was 220°C and that of the ionizing chamber 200°C.

Paper chromatography (PC) was performed on Whatman No. 1 and 3MM papers, using ethyl acetate=acetic acid=water (18:7:8) (solvent D); or n-butanol=ethanol=water (10:3:3) (solvent E). Sugars were detected with anilinium hydrogen phthalate. The mobilities (R_{Cel}) are expressed relative to that of D-cellobiose. The retention times (T) of the methylated alditol acetates are given relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol.

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Cellulose content was determined by the method of Updegraff (1969), lignin by Klason (1967), uronic acids by the modified method of Bitter and Muir (1962), carbohydrates by the phenol-sulphuric acid method (Dubois *et al.*, 1956) and acetyl content by GLC with β-D-glucose penta-acetate as standard (column (c)) (Meier, 1961).

Proton-decoupled ^{13}C-NMR spectra (75·46 MHz) were recorded in the pulsed Fourier transform (F.t.) mode with a Bruker AM-300 spectrometer. The spectral width was 17 kHz, pulse width 12 μs, and the number of data points 16 k. Methanol was used as the internal standard (50·15 ppm).

Sedimentation analysis (at various concentrations) was performed with an MOM 10 ultracentrifuge. Photographs were taken at intervals of 6 min (50 000 r.p.m.). The partial specific volume (0·615 ml/g) of the polysaccharide was determined from the sum of the specific volumes of monomers (Gibbons, 1972).

The molecular mass determination was carried out with a Knauer osmometer at 128 sensitivity and 37°C, using a universal probe (25–60°C).

Gel filtration was effected on a column (1·3 × 150 cm) of Bio-Gel P-2, with maltose, maltotri-hexaose (Serva) as standards. Water containing 0·02% sodium azide was used as the eluent. Carbohydrate content was detected with a differential refractometer, RIDK 101.

The wood sawdust was hydrolysed by 72% (w/w) sulphuric acid for 1 h diluted to 8% (w/w) and heated at 100°C for 4 h. The hydrolysate was neutralized with barium hydroxide, filtered, treated using a mixed-bed ion exchanger V (Merck) and concentrated. The polysaccharides were treated with 90% (w/w) formic acid for 7 h at 100°C and the acid was evaporated. Sugars were analysed as alditol trifluoroacetates by GLC. The oligosaccharides were hydrolysed with 90% (w/w) formic acid for 2 h at 100°C, identified by PC (solvent E) and quantified densitometrically.

Isolation and fractionation of galactoglucomannan

Sawdust prepared from the trunk of *P. monilifera* was exhaustively extracted with chloroform:methanol (2:1 w/w) and then air dried. The lipid-free material contained the following percentages; cellulose (54·0), lignin (20·6), uronic acid (4·9), O-acetyl (3·2), galactose (0·3), glucose (50·9), mannose (1·7), xylose (16·1) and rhamnose (0·8).

The holocellulose obtained by sodium chlorite treatment (Klauditz, 1957) (110 g, 90·4% of dry wood) was extracted (2 h, 25°C under N_2) with aqueous 15% potassium hydroxide (1500 ml) to give a hemicellulose fraction (34·9 g, 28·7% of dry wood). The water soluble part of this fraction representing 14·4% of dry wood was (4-O-methyl-D-glucurono)-D-xylan (Karácsonyi and Kubačková, 1969).

The residual material was then extracted with aqueous 10% sodium hydroxide containing 4% boric acid (1000 ml) for 4 h at 25°C under N_2. The extract was neutralized with acetic acid and the precipitate was separated by centrifugation, washed with ethanol and acetone, then dried *in vacuo* to yield the polysaccharide P1 (3·59 g). From the supernatant, after dialysis and freeze-drying, the polysaccharide P2 (6·2 g) was obtained. The yields and composition of polysaccharides are detailed in Table 1.

The solution of polysaccharide P2; 2% w/v in 0·01 M acetate buffer (200 ml; pH 5·0) was incubated with xylanase from *Trametes hirsuta* (Kubačková *et al.*, 1979) (10 mg, 1·5 μkat) at 40°C for 48 h. The reaction was terminated by heating in a water bath (100°C, 15 min). The reaction mixture was centrifuged and the supernatant obtained dialysed (H_2O, 48 h), and freeze-dried. The purified polysaccharide P3 (2·2 g) (Table 1) was used for further investigation. Sedimentation analysis ($S_{o_{20}}$ 1·28 S) and electrophoretic mobility (5·03 × 10^{-5} cm^2/V/s) confirmed the homogeneity of the polysaccharide.

Partial hydrolysis

The polysaceharide P3 (700 mg) was treated at 100°C for 2 h, first with aqueous 10% formic acid (20 ml) and then with aqueous 20% formic acid. The hydrolysate after each treatment was concentrated and the part to be hydrolysed further was precipitated with ethanol. The low-molecular fragments (480 mg), after fractionation on a column (1·3 × 150 cm) of Bio-Gel P-2 with

Table 1. Glycosyl residue composition of polysaccharides

Polysaccharide	Yield[a] (%)	[α] D (degree)	Mole ratio of monosaccharides					
			Gal	Glc	Man	Ara	Xyl	Rha
P1	2·9	−72·4[b]	tr[c]	1·9	3·6	0·2	1·0	tr[c]
P2	5·2	−45·0	0·4	1·1	1·7	-	1·0	-
P3	2·9	−10·2	1·0	4·1	9·7	-	tr[c]	-

[a]Based on the dry mass of wood.
[b](c, 1·0 in 0·5 NaOH).
[c]tr = trace.

Table 2. Oligosaccharides derived from galactoglucomannan on acid hydrolysis

Oligosaccharide	R_{Cel}[a]	[α] D (degrees)	D.p.[b]	Mole (%)		
				Gal	Man	Glc
1	0·7	+26·9	2		48·0	52·0
2	1·0	+31·0	2			100·0
3	1·1	−11·8	2		100·0	
4	1·6	+15·0	2		48·0	52·0
5	0·69	−20·0	3		100·0	
6	0·95	−11·0	3		67·0	33·0
7[c]	0·33	+73·0	3	30·0	34·0	36·0

[a]Paper chromatography using solvent E.
[b]Degree of polymerization determined by gel filtration on Bio-Gel P-2.
[c]Borohydride reduced compound 7 and gave galactose and mannose in the mole ratio 1:1.

water, gave galactose, mannose, di- and tri-saccharides, and a mixture of higher oligosaccharides (degree of polymerisation (d.p. 4–6). Preparative PC on Whatman No. 3MM paper yielded components with R_{Cel} 0·33, 0·69, 0·73, 0·95, 1·0, 1·1 and 1·6 (solvent E) (Table 2).

Oligosaccharides 1–7 (5–10 mg) were each methylated with methyl iodide (2 ml) and sodium hydride (20 mg) in N,N-dimethylformamide (2 ml). The products were identified by GLC-MS (Jansson *et al.*, 1976) using column (b) (Tables 2 and 3).

Methylation

The polysaccharide P3 (20 mg) was methylated once by the Hakomori (1964) method and twice by the Purdie method (Hirst & Percival, 1965) to give a product which had negligible i.r. absorption for hydroxyl. The per-methylated polysaccharide was hydrolysed and the partially methylated saccharides were identified as the corresponding alditol acetates by GLC-MS (column (b)). The results are given in Table 3.

RESULTS AND DISCUSSION

The lipid-free poplar sawdust was delignified (Klauditz, 1957) and the holocellulose then extracted with aqueous potassium hydroxide solution to give a mixture of polysaccharides from which (4-O-methyl-D-glucurono)-D-xylan (14·4% of the wood) was obtained and structurally characterized (Karácsonyi & Kubačková, 1969).

The residue, after xylan isolation, was extracted with aqueous sodium hydroxide containing boric acid. For isolation of the galactoglucomannan from this hemicellulose fraction only two purification steps were used. Neutralization of the alkaline solution yielded an insoluble, precipitated polysaccharide, P1, representing 2·9% of the wood, and a soluble polysaccharide, P2 (5·2% of the wood). Polysaccharide P1 contained glucose, mannose and xylose, in addition to small amounts of galactose and arabinose, while in polysaccharide P2 arabinose was absent. The mole ratios of the polysaccharide components as determined by GLC are given in Table 1. Treatment of the crude galacto-

Table 3. Methylated sugars from hydrolysate of methylated galactoglucomannan (GGM) and derived oligosaccharides 1–7

Sugar (as alditol acetates)	T[a]	Mole (%)								Linkage indicated
		GGM	1	2	3	4	5	6	7	
2,3,4,6-Me_4-Man[b]	1·03	1·4	49·0		49·5		32·0			D-Manp-(1→
2,3,4,6-Me_4-Glc	1·00	Tr[c]		48·00		49·4		35·0		D-Glcp-(1→
2,3,4,6-Me_4-Gal	1·10	7·6							30·0	D-Galp-(1→
2,3,4 -Me_3-Man	1·20								33·0	→6)-D-Manp-(1→
2,3,6 -Me_3-Man	1·28	60·4			50·5	50·6	68·0	65·0		→4)-D-Manp-(1→
2,3,6 -Me_3-Glc	1·39	22·4	51·0	52·0					37·0	→4)-D-Glcp-(1→
2,3 -Me_2-Man	1·65	5·3								→4,6)-D-Manp-(1→
2,3 -Me_2-Glc	1·80	2·9								→4,6)-D-Glcp-(1→

[a]Retention time relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylglucitol on column (b).
[b]2,3,4,6-Me_4-Man = 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylmannitol, etc.
[c]Tr = trace.

glucomannan (fraction P2) with *Trametes hirsuta* xylanase (Kubačková *et al.*, 1979) was found to be the most efficient purification procedure.

The purified polysaccharide PC, $[\alpha]_D$ − 10·20° (c, 1% w/v concentration of the sample in H_2O) was homogeneous in electrophoresis (5·03 × 10^{-5} cm^2/V/s) and ultracentrifugation (So_{20} 1·28 S) and consisted of galactose, glucose and mannose in the mole ratio 1:4·1:9·7. The molecular mass determined by osmometry was Mn 1·15 × 10^4 (d.p. 71).

The yield of galactoglucomannan from poplar (2·9% of the wood) is lower than that generally found in the wood of conifers (Timell, 1965), but higher than that reported for *Populus tremuloides* bark (Jiang & Timell, 1972), which, according to our knowledge is the only polysaccharide of this type isolated from deciduous trees. The low content of galactoglucomannan in poplar wood may be explained by the fact that the predominant component of hardwood (unlike softwood) hemicelluloses is (4-O-methyl-D-glucurono)-D-xylan.

Partial acid hydrolysis of galactoglucomannan under mild conditions gave a mixture of component sugars, di-, and tri-saccharides 1–7, and the fraction of higher oligosaccharides which were resolved on Bio-Gel P-2 (Fig. 1) and by paper chromatography. The homogeneous oligomers 1–7 were identified by methylation analysis, mass spectrometry and ^{13}C-NMR spectroscopy as β-D-Manp-(1→4)-D-Glcp (1), β-D-Glcp-(1→4)-D-Glcp (2), β-D-Manp-(1→4)-D-Manp (3), β-D-Glcp-(1→4)-D-Manp (4), β-D-Manp-(1→4)-β-D-Manp-(1→4)-D-Manp (5), β-D-Glcp-(1→4)-β-D-Manp-(1→4)-D-Manp (6), and α-D-Galp-(1→6)-β-D-Manp-(1→4)-D-Glcp (7) (Tables 2 and 3). The mixture of higher oligosaccharides (d.p. 4–6) was not further studied.

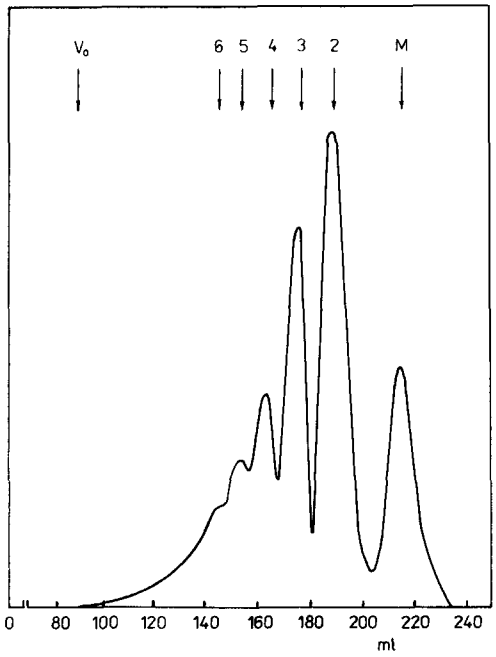

Fig. 1. Gel filtration on Bio-Gel P-2 of the oligosaccharides from galactoglucomannan hydrolysate. V_0, void volume: 2–6, standard compounds of malto-oligosaccharides d.p. 2–6: M, D-mannose.

The partially methylated saccharides obtained by hydrolysis of the methylated galactoglucomannan were identified as the corresponding alditol acetates by GLC and GLC-MS (Jansson *et al.*, 1976). The results are listed in Table 3.

Identification of the compounds 1–7 and the results of methylation analysis showed that only 4-linked mannopyranosyl (60·4%) and 4-linked glucopyranosyl residues (22·4%), presumably distributed at random, formed the (1→4)-linked polysaccharide backbone, having single stubs of (1→6)-linked galactopyranosyl residues attached to both glucosyl and mannosyl residues. The properties of the 4,6-linked glucosyl and 4,6-linked mannosyl residues in the mole ratio approximately 1:1·8 compared with the original polysaccharide composition (glucose and mannose 1:2·4) suggested slightly preferable substitution of the mannosyl residues in the galactoglucomannan structure. The mole ratio of the terminal mannopyranosyl residues to other components in the molecule of polysaccharide 1:70 indicated a degree of polymerization of the galactoglucomannan of approximately 70. This result is in good agreement with the value of 71 determined osmometrically and also confirmed the linearity of the backbone.

The structural features of galactoglucomannan outlined above were confirmed by ^{13}C-NMR spectroscopy. The ^{13}C-NMR spectrum of the polysaccharide exhibited signals in the anomeric region at 103·7 ppm and 101·3 ppm, respectively, attributable to the β-D-glucopyranosyl and βD-mannopyranosyl residues, while the unambiguous identification of the anomeric form of the galactosyl residues was not possible from the NMR data of the polysaccharide owing to their low concentration and coincidence with the high intensity signals of C-1 atoms of β-mannopyranosyl residues (Table 4). The α-pyranose form was

Table 4. ^{13}C-NMR data for *Populus monilifera* H. galactoglucomannan

	Chemical shifts (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
β-Glcp-(1-CH_3[a]	104·0	74·1	76·8	70·6	76·8	61·8
β-Manp-(1-CH_3[a]	101·3	70·6	73·3	67·1	76·6	61·4
α-Galp-(1-CH_3[a]	100·1	69·2	70·5	70·2	71·6	62·2
→4)-Glcp-(1-	103·7	74·1	75·2	79·7	75·9	61·8
→4)-Manp-(1-	101·3	71·3	72·7	77·7	76·2	61·8
α-Galp-(1-	[b]	69·2	70·5	69·5	72·0	63·0

[a] According to Bradbury & Jenkins (1984).
[b] Presumably overlapped by the signal of C-1 of 4-linked β-mannopyranosyl residues.

assigned to the galactosyl residues from C-1 resonances of compound 7 compared with data from the literature (Bradbury & Jenkins, 1984). The tri-saccharide showed chemical shifts at 99·4, 101·3, 96·8 and 94·2 ppm which were assigned to the α-D-galactopyranosyl, β-D-mannopyranosyl, and α,β-D-glucose residues, respectively.

The integrated intensities of the signals for both anomeric carbons of D-mannopyranose and D-galactopyranose residues to that of the D-glucopyranose (2·6:1) correspond with the results of chemical analysis (2·4:1). In agreement with the results of chemical analysis, the signals of the C-4 resonances indicated that the polymer consisted of 4-linked mannopyranosyl and 4-linked glucopyranosyl residues in the galactoglucomannan structure (Table 4). In addition to the major signals the ^{13}C-NMR spectrum of the polysaccharide also displayed low intensity signals pointing to substitution at C-6 of these units, as well as resonances due to carbons of galactosyl residues. The signals at 68 ppm may be due to the C-6 carbons of 4,6- linked D-glucopyranose and 4,6-linked D-mannopyranose.

These results suggest that the galactoglucomannan from poplar wood consists of a main chain of (1→4)-linked β-D-mannopyranosyl and β-D-glucopyranosyl residues distributed at random, having single stubs of (1→6)-linked α-D-galactopyranosyl residues attached to both mannosyl and glucosyl residues, but with slightly preferable substitution of the mannosyl residues.

The galactoglucomannans isolated from different species of plants indicated essentially the same structural features which differed only in details, such as the chemical composition of constituent saccharides, their sequential distribution, the degree of branching and the substitution patterns. The structure of galactoglucomannan from poplar resembled that commonly found in a number of softwoods including *Tsuga canadensis, Picea engelmanni, Abies amabilis* and *Populus tremuloides* bark (Dey, 1980). With respect to relative sugar composition, degree of polymerization and the galactose content, poplar galactoglucomann is very similar to polysaccharide from the wood of *Pinus resinosa* (Timell, 1970).

The presence of galactoglucomannan in the primary cell walls of actively growing cells suggests that the derived fractions of these polymers may have biological activity as has been evidenced for fragments of xyloglucan (Aldington *et al.*, 1991). The structure-activity relationship for oligosaccharides 4–7 and the higher oligomers are at present under investigation (D. Lišková, unpublished data).

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