A GALACTOGLUCOMANNAN FROM THE STEM TISSUES OF THE AQUATIC MOSS *Fontinalis antipyretica*

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ABSTRACT

A galactoglucomannan[1], isolated from the stem tissues of the aquatic moss *Fontinalis antipyretica*, has $[\alpha]_D^{18}$ $-11°$ and, on hydrolysis, yields galactose, glucose, and mannose in the molar ratios of 10:10:37. It has a main chain of β-(1→4)-linked D-mannopyranosyl and D-glucopyranosyl residues, and there is no evidence that the latter residues are contiguous. D-Galactopyranosyl and D-mannopyranosyl residues are attached to C-6 of D-mannopyranosyl, and to a lesser extent of D-glucopyranosyl, residues of the main chain. The methylated galactoglucomannan has a d.p. of 33–37 by ebulliometry, and the parent hemicellulose has ratios of galactose, glucose, and mannose residues of 10:11:52. The galactoglucomannan is very similar to many found in softwoods[2,3], and is structurally similar to that isolated from bracken[4], although the sugar ratios (1:15:60) are different. The isolation of the galactoglucomannan from a *Bryophyte* is of chemotaxonomic interest.

INTRODUCTION

The structures of many hemicelluloses have been established, but most of these hemicelluloses were lated from the lignified tissues of plants of the *Spermatophytae*[2,3]. There has been little work on the polysaccharides from the plants of other families, although such studies could be of chemotaxonomic interest. Studies have been reported of galactoglucomannans and acidic xylans isolated from the *Pteridophytae* cinnamon fern (*Osmunda cinnamonea*)[5] and bracken (*Pteridium aquilinum*)[4,6]. A galactoglucomannan and an arabinogalactan have been isolated from the *Bryophyte Fontinalis antipyretica*, and the present paper reports on structural studies of the former hemicellulose.

RESULTS AND DISCUSSION

The submerged, aquatic moss *Fontinalis antipyretica* was harvested from the River Dee (Aberdeenshire), and the enzymes in the tissues were inactivated with

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boiling ethanol. It has been stated that mosses lack, or may lack, lignin but, in the present instance, it was possible to extract a significant proportion of the polysaccharides only after the wiry and woodlike stem tissues had been treated with acid–chlorite[7]. The terms holocellulose, α-cellulose, and hemicellulose normally describe polysaccharide materials isolated from formerly lignified, land-plant tissues; here they are extended to cover the materials obtained by analogous procedures from the moss tissues. Milled holocellulose was treated[8] twice with methyl sulphoxide at 20° and then with water at 60°. The two methyl sulphoxide extracts gave materials which, on hydrolysis, yielded galactose, glucose, mannose, arabinose, and xylose in the molar ratios of *ca.* 13:10:40:6:4. The material from the first extract contained methoxyl (0.8%) and acetyl groups (5.2%). No precipitate was obtained on addition of an excess of ethanol to the acidified, aqueous extract.

Holocellulose was treated, successively, a number of times with 5, 10, and 20% aqueous potassium hydroxide under nitrogen. The hemicellulosic materials, isolated from each extract by conventional procedures, were combined because they were similar in sugar residue composition. The resulting material accounted for 20% of the holocellulose. A galactoglucomannan was isolated by dissolving this hemicellulosic material in 5% aqueous potassium hydroxide followed by graded percipitation with barium hydroxide[9,10]. Application of other fractionation methods provided no evidence that the product was a mixture of hemicelluloses. The galactoglucomannan was homogeneous on electrophoresis in 0.1M sodium borate, whereas the unfractionated hemicellulosic material contained three components.

The galactoglucomannan was water soluble and had $[\alpha]_D^{18}$ −11°. On acidic hydrolysis, it gave galactose, glucose, mannose, and xylose in the molar ratios of 10:10:37: trace. The methylated galactoglucomannan (OMe, 43.4%), which displayed no band in its infrared spectrum attributable to hydroxyl groups, had a d.p. of 33–37 (ebulliometry in 8% butanone). A methanolysate contained the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, and unidentified components that were probably di-*O*-methylhexoses. After methanolysis and hydrolysis of the methylated galactoglucomannan, the products were identified by conventional procedures and their molar proportions estimated from the weights recovered after fractionation. The hydrolysate contained 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-mannose, and 2,3-di-*O*-methyl-D-glucose in the molar ratios of 100:27:428:102:63:10, respectively. Estimations based on g.l.c. peak-area measurements on sub-fractions gave molar ratios of 100:25:400:96:55:10. The former values are used in subsequent calculations. There was a smaller proportion of di-*O*-methylhexoses than that required to account for the amounts of tetra-*O*-methylhexoses present and this was probably caused by slight losses during the paper-chromatographic sub-fractionation of mixtures of small quantities of the di-*O*-methylhexoses. On mild, acid hydrolysis of the galactoglucomannan, the galactose was stripped off completely without any accompanying release of glucose and mannose. This release

of galactose, and the occurrence of 2,3,4,6-tetra-*O*-methyl-D-galactose in the hydrolysate of the methylated galactoglucomannan, proved the presence of D-galactopyranosidic residues in terminal, non-reducing positions. Partial hydrolysis of the galactoglucomannan, by acid and by use of a hemicellulase, gave β-(1→4)-linked di-, tri-, and tetra-saccharides of D-mannose, and 4-*O*-β-D-glucopyranosyl-D-mannose, but no cellobiose.

The methylation and partial hydrolysis studies are compatible with a hemicellulosic material that had been converted into a methylated galactoglucomannan having d.p. *ca.* 35 and an average of 20.5 D-mannopyranosyl and 4.9 D-glucopyranosyl residues linked β-(1→4). There was no evidence of contiguous D-glucose residues in the main chain which contained, on average, 5.2 D-galactopyranosyl and 1.4 D-mannopyranosyl non-reducing, terminal residues, one of which was the end-residue of the main chain. The values are based on the proportions of tetra-*O*-methyl-D-hexoses recovered on fractionation. The side-residues were attached to C-6 of ~4.8 D-mannopyranosyl and 0.8 D-glucopyranosyl residues in the main chain. Two possibilities are worthy of consideration in future studies. One is that the main chain may be terminated by a D-mannopyranosyl residue and that there may be only D-galactopyranosyl, terminal side-residues. The other is that the terminal D-galactopyranosyl residues may be attached solely to D-mannopyranosyl residues on the main chain, and that the terminal D-mannopyranosyl residues are attached solely to D-glucopyranosyl residues of the main chain.

The ratios of galactose:glucose:mannose in a galactoglucomannan hydrolysate were 10:10:37 and there was a trace of xylose, whereas, in the methylated galactoglucomannan, the ratios of the methylated galactose, glucose, and mannose residues were 10:11:52, and no derivatives of xylose were detected. Invariably, losses of polysaccharides occur during methylation procedures, and it appears that, in the present instance, a hemicellulosic material richer in galactose and glucose residues was selectively lost during fractionation. It is believed that the loss is not due to selective cleavage of the galactose residues during methylation. The ratio of non-reducing, terminal side-residues to others was ~1:5 in the methylated galactoglucomannan, but periodate oxidation, including Smith-degradation studies, of the galactoglucomannan indicated a lower degree of branching.

The material extracted from the holocellulose by methyl sulphoxide contained 5.2% of acetyl groups, indicating that the moss-stem galactoglucomannan was probably partially acetylated in its native state as are the galactoglucomannans from certain coniferous woods.

The molar ratios of the galactose, glucose, and mannose residues in the galactoglucomannan[11] from *F. antipyretica* were 10:10:37. From the woods of many species of *Gymnospermae*, two types of galactoglucomannans have been isolated that are similar to the galactoglucomannan from the moss and have galactose:glucose:mannose ratios of 10:10:30 and 1:10:30. A galactoglucomannan from the fern *Pteridium aquilinum*[4] had the above sugar residues in the ratios of 1:15:60 and the derived, methylated galactoglucomannan had a d.p. of 25–30 and ratios of methylated or

partly methylated galactose, glucose, and mannose residues of *ca.* 1:10:33. It may be that two types of galactoglucomannan were present in the moss hemicellulosic material, as in the material from *Gymnospermae*, and that the one richer in galactose was lost by inadvertent fractionation accompanying methylation.

An arabinogalactan has been isolated from *F. antipyretica*[1,12] and a low proportion of a hemicellulose containing xylose residues is evidently present also. Arabinogalactans and low proportions of xylans occur in softwoods and members of the *Larix* genus[11], and it is believed that the present work on the hemicellulose of a *Bryophyte* is of chemotaxonomic interest.

EXPERIMENTAL

Paper chromatography was performed on Whatman No. 1 paper. R_C, R_{Glc}, and R_{TMG} values are mobilities relative to cellobiose, D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose, respectively. The irrigants were ethyl acetate–pyridine–water (*A*, 360:100:115; *B*, 2:1:2; *C*, 10:4:3), ethyl acetate–acetic acid–formic acid–water (*D*, 18:3:1:4), ethyl acetate–acetic acid–water (*E*, 3:1:3; *F*, 3:1:1), 1-butanol–pyridine–benzene–water (*G*, 5:3:1:3), butanone–water (*H*, 2:1), 1-butanol–ethanol–water (*J*, 4:1:5), and ethyl acetate–pyridine–saturated, aqueous sodium acetate (*K*, 360:100:115). Unless otherwise stated, the irrigants for methylated sugars were *F*, *H*, and *J*, and for oligosaccharides *B*, *C*, and *G*; the respective mobilities are given in these sequences later. Detection on chromatograms was by methanolic *p*-anisidine hydrochloride. Irrigant *H* was used for t.l.c. of methylated sugars on silica gel. Sugars in hydrolysates were determined by the phenol–sulphuric acid[13] or *p*-anisidine hydrochloride–stannous chloride methods[14]. Gas–liquid chromatography (g.l.c.) was performed on a Pye-Argon chromatograph, using columns of acid-washed Celite (100–120 mesh) coated with either 20% Apiezon M (*G1*), 15% poly(butane-1,4-diol succinate) (*G2*), or 10% *m*-bis(*m*-phenoxyphenoxy)benzene (*G3*). The retention times (*T*) are relative to that of methyl 2,3,4,6-tetra-*O*-methyl-*β*-D-glucopyranoside. A Shandon high-voltage electrophoresis apparatus was used with Whatman GBH-1 glass-fibre paper, and low-voltage electrophoresis of monosaccharides was performed on Whatman No. 1 paper. In each case, 0.1M borax buffer was used, and mobilities (M_{Glc}) are expressed relative to that of D-glucose. Thin-layer electrophoresis of polysaccharides was performed on glass plates (20 × 5 cm) coated with silica gel and wetted with 0.1M cetyltrimethylammonium borate. Components were detected with potassium permanganate–sulphuric acid or iodine vapour. Polysaccharides (2 × 10 mg) were hydrolysed in sealed tubes with 0.5M sulphuric acid for 6 h at 100°, and hydrolysates were neutralised with barium carbonate. Infrared spectra were determined with a Perkin–Elmer 137 Infracord instrument. Solvents were removed at, or below, 35° under reduced pressure. After being washed with iced water, polysaccharides were washed with ethanol and ether and dried ($\leqslant 35°$).

Isolation of the galactoglucomannan. — Submerged *Fontinalis antipyretica* was collected, during September and October 1962, from the River Dee (Aberdeenshire).

The plantstuff was washed to remove grit; there was no algal or other contamination. The moss was immersed in boiling ethanol to inactivate enzymes and then air-dried. The material (700 g) was macerated in ethanol, and leaf and stem material was separated by flotation. The stem material (430 g), on treatment with acid–chlorite[7], gave a white holocellulose (375 g). Samples (3 × 100 g) of the holocellulose were each treated with 5, 10, and 24% potassium hydroxide (1 × 2 l; 2 × 1 l and 3 × 2 l; and 3 × 2 l, respectively) under nitrogen. The extracts were neutralised with acetic acid and, on the addition of an excess of ethanol, a precipitate formed in each case. Hydrolysates of samples of the precipitates were similar, and so the hemicellulosic materials (61 g) were combined.

Hydrolysis of a thoroughly washed sample (50 mg) of the α-cellulose at 100° with M sulphuric acid (1 ml) gave (paper chromatography) glucose and a little mannose.

The hemicellulosic material (60.4 g) was dissolved in 5% aqueous potassium hydroxide (1.6 l), and insoluble material (457 mg) was removed. Saturated barium hydroxide (1 l) was added dropwise, and the precipitate was collected, washed with ice–water (200 ml), and dissolved in 2M acetic acid (400 ml), and ethanol (1.2 l) was added. The resulting white precipitate (*P1*, 36.5 g) was collected and dried[9,10]. The mother, and wash, liquors were combined and, after acidification with 2M acetic acid, ethanol (3 vol.) was added and the precipitate (*S1*, 23.1 g) was collected. Paper-chromatographic examination of hydrolysates of *P1* and *S1* showed a markedly higher ratio of hexose to pentose in the former. Sub-fractionation with barium hydroxide gave fractions *P2–P5* and *S2–S5*. Hydrolysates of the various *P* fractions contained galactose, glucose, and mannose in the molar ratios of 10:10:37, and a trace of xylose. Unsuccessful attempts were made to sub-fractionate fraction *P5* by separate treatments with Fehling's solution, with cupric acetate and ethanol[15], by ultrafiltration[16], and by chromatography on a column of cellulose. On electrophoretic examination (3 volts/cm, 3 h) of the parent hemicellulosic material on glass paper, three components were detected. The galactoglucomannan travelled as a discrete zone. The galactoglucomannan had $[\alpha]_D^{18}$ −11° (*c* 2.2, M sodium hydroxide).

Periodate oxidation of the galactoglucomannan. — Aqueous solutions (50 ml) of two samples (98 and 86 mg) of the galactoglucomannan were oxidised for 10 days in the dark with 0.4M sodium metaperiodate. The mmoles of oxidant reduced per anhydrohexose residue were 690 (24 h), 970 (32), 1050 (53), 1070 (96), 1080 (120), 1080 (144), 1080 (173), and 1090 (200), giving an extrapolated value of 1080 at zero time. The corresponding mmoles of formic acid released were 97 (33 h), 109 (96), 110 (120), 111 (130), 113 (173), and 115 (200), giving an extrapolated value of 107 at zero time. No formaldehyde was released up to 96 h (chromotropic acid method). A sample (157 mg) of the galactoglucomannan was oxidised in the dark for 21 days with 0.2M sodium metaperiodate (100 ml), 0.2M barium acetate (50 ml) was then added, and the insoluble salts were removed. The solution was deionized with Amberlite IR-120 (H^+) and IR-40 (OH^-) resins, and the water was removed. The resulting syrup (112 mg) was redissolved in water, and treated for 3 days with sodium borohydride.

After decomposition of the excess of borohydride with Amberlite IR-120 (H^+) resin, the water was evaporated, and borate was removed as methyl borate by distillation of methanol from the residue. The polyalcohol was treated with 0.5M sulphuric acid (15 ml; 6 h at 100°), and, after neutralisation with barium carbonate, the hydrolysate was concentrated and examined by paper chromatography (irrigants *A* and *B*). The main components were glycerol and erythritol, and there were traces of glycolaldehyde, glucose, and mannose. A sample of the polyalcohol hydrolysate was esterified with acetic anhydride–sodium acetate. Examination of the products by g.l.c. (column *G1*) showed the presence of components having *T* values corresponding to erythritol tetra-acetate and glycerol triacetate in the molar ratio of 146:10. One of the components from the acetylated hydrolysate of the polyalcohol was crystallised from ether–light petroleum (b.p. 60–80°) and gave erythritol tetra-acetate, m.p. 87–88°.

Methylation and degradation of the galactoglucomannan. — A sample (4.1 g) of the galactoglucomannan was methylated under nitrogen by treatments with 40% aqueous potassium hydroxide and methyl sulphate. The partially methylated product (3.82 g; OMe, 27.4%) was dissolved in *N,N*-dimethylformamide (20 ml) and, after four treatments[17,18] with silver oxide (12 g) and methyl iodide (10 ml), it gave a product having OMe, 43.4% (theoretical maximum, 45.6%). There was no hydroxyl band in the infrared spectrum. The molecular weight (ebulliometry in 8% butanone[19]) was 6,700–7,500, corresponding to a d.p. of 33–37.

The methylated galactoglucomannan (1.05 g) was boiled under reflux with anhydrous 4% methanolic hydrogen chloride (30 ml) for 1 day. G.l.c. (phases *G1* and *G2*) then showed peaks (w, weak; m, medium; s, strong) corresponding to the methyl glycosides of 2,3,4,6-tetra-*O*-methylmannose (*T* 1.15w and 1.43w), 2,3,4,6-tetra-*O*-methylgalactose (1.35m and 180m), 2,3,6-tri-*O*-methylglucose (1.48wm, 1.80m and 3.52wm, 4.80wm), 2,3,6-tri-*O*-methylmannose (2.06s and 4.99s), di-*O*-methylhexoses (?; 2.66wm, 3.04wm; and 6.70wm, 7.64wm, 8.25wm). A component having a *T* value of 2.52w was noted on g.l.c. on phase *G2*.

The methanolysate was hydrolysed with M hydrochloric acid, the hydrolysate was neutralised with silver carbonate, and silver ions were removed as the sulphide. The solution was concentrated, and the syrupy residue (921 mg) was dissolved in ethanol and fractionated by chromatography on acid- and water-washed carbon and Celite (1:1) using an aqueous ethanol gradient (2→30%; 5 l). Successive fractions had weights and R_{TMG} values (irrigant *H*): 42 mg (0.26, 0.39), 39 mg (0.26, 0.68, 0.39), 362 mg (0.69), 151 mg (0.69, 0.86), 48 mg (0.86), 58 mg (0.86, 0.93), 82 mg (0.93, 1.00), and 26 mg (1.00). Mixed fractions were subfractionated by paper chromatography and gave pure components *1–6*.

Component *1* (32 mg) was a syrup, $[\alpha]_D^{18}$ +8° (*c* 0.5, methanol). It was indistinguishable from 2,3,4,6-tetra-*O*-methyl-D-mannose on paper chromatograms, t.l.c., and reverse-phase chromatography on paper impregnated with methyl sulphoxide and irrigated by isopropyl ether (two spots were noted in each case). On demethylation, the component gave mannose only. The aniline derivative of component *1* had m.p. 142–143° [from ether–light petroleum (b.p. 60–80°)]. Methyl glycosidation of *1*

gave a derivative having *T* 1.17 on phase *G1*, and 1.39 on phase *G2*, corresponding to the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-mannose[20].

Component *2* (119 mg) was a syrup, $[\alpha]_D^{19}$ +96° (*c* 0.6, water), which was indistinguishable (paper chromatograms and t.l.c.) from 2,3,4,6-tetra-*O*-methyl-D-galactose. The aniline derivative had m.p. 191–192° [from ether–light petroleum (b.p. 60–80°)]. The product obtained on methyl glycosidation of *2* had *T* 1.38 and 1.78 on phases *G1* and *G2*, respectively, corresponding to the methyl 2,3,4,6-tetra-*O*-methyl-D-galactosides[20].

Component *3* (114 mg) was a syrup, $[\alpha]_D^{19}$ +70° (*c* 1.0, water). It was indistinguishable by paper chromatography and t.l.c. from 2,3,6-tri-*O*-methyl-D-glucose. On demethylation, it gave only glucose. The derived di-*p*-nitrobenzoate had m.p. 189–190° (from methanol). The derived methyl glycosides of *3* had *T* 1.48, 1.79, and 2.12 on phase *G1*, and 3.52, 4.76, and 5.02 on phase *G2*. These values indicate the presence of methyl 2,3,6-tri-*O*-methyl-D-glucopyranosides contaminated by a small proportion of methyl 2,3,6-tri-*O*-methyl-D-mannopyranosides[20].

Component *4* (478 mg) was a syrup, $[\alpha]_D^{20}$ −12° (*c* 1.0, water), which was indistinguishable by paper chromatography and by t.l.c. (irrigant *A*) from 2,3,6-tri-*O*-methyl-D-mannose. On demethylation, it gave only mannose. The di-*p*-nitrobenzoate of *4* had m.p. 187–188° (from methanol). Methyl glycosidation gave a single product having *T* 2.08 and 4.99 on phases *G1* and *G2*, respectively, corresponding to a methyl 2,3,6-tri-*O*-methyl-D-mannoside[20].

Component *5* (12 mg) was a syrup which was indistinguishable from 2,3-di-*O*-methyl-D-glucose on paper chromatograms, on t.l.c. plates, and on paper electrophoretograms (M_{Glc} 0.18). Demethylation gave glucose, and methyl glycosidation gave two components having *T* 2.51 and 3.25 on phase *G3*, corresponding to methyl 2,3-di-*O*-methyl-D-glucosides[20].

Component *6* (66 mg) was a brown syrup containing components with R_{TMG} values of 0.69, 0.39, and 0.56 on paper chromatograms, 0.26 on a t.l.c. plate, and M_{Glc} 0.17 on an electrophoretogram. Demethylation gave mannose, and methyl glycosidation gave a single product with *T* 3.42 on phase *G3*, corresponding to methyl 2,3-di-*O*-methyl-D-mannoside[20].

Partial hydrolysis of the galactoglucomannan. — A sample (28 mg) of the galactoglucomannan was treated with boiling 50mM formic acid (20 ml, 16 h). Aliquots (1 ml) were withdrawn at intervals, and formic acid was removed by codistillation with water. After hydrolysis of the formic esters by heating in 25mM sulphuric acid (5 ml), the hydrolysates were neutralised with barium carbonate and examined by paper chromatography (irrigants *A* and *B*). Galactose was liberated during the first 10 h, glucose after 13 h, and mannose after 17 h; oligosaccharides having R_{Glc} values (irrigant *B*) from 0.2–0.7 were also noted.

A sample (1.51 g) of the galactoglucomannan was shaken with water (50 ml) and, after removal of the insoluble material (86 mg), a 10% solution of Hemicellulase (Koch–Light) (30 ml) was added, and the solution was dialysed at 35° for 12 h against water (14 × 1 l). The degraded polysaccharide in the dialysate was hydrolysed

with acid to give galactose, glucose, mannose, and a trace of xylose. Concentration of the diffusate gave a syrup (1.31 g) which was dissolved in water (40 ml) and fractionated on charcoal–Celite (1:1). Elution with water gave a syrup (421 mg) containing (paper chromatography) galactose, glucose, mannose, and xylose in the molar ratios of 10:8:20:3. Oligosaccharides (583 mg) were eluted by an aqueous ethanol gradient (2→6%, 5 l). By sub-fractionation on paper chromatograms using irrigants *B*, *C*, and *G*, seven chromatographically and electrophoretically homogeneous oligosaccharides were isolated. Another sample of the galactoglucomannan in water was treated with Hemicellulase (Koch–Light), and oligosaccharides were removed by the dialysis technique. The diffusate was found to contain oligosaccharides corresponding to oligosaccharides *1–4* obtained by acid hydrolysis.

Study of oligosaccharides in acidic hydrolysates[4]. — A sample (2–4 mg) of each oligosaccharide was hydrolysed before and after reduction with sodium borohydride (10 mg) in water (5 ml). The neutralised solution was concentrated and deionised, and the borate removed as described earlier. A sample (4–10 mg) of each oligosaccharide was methylated[17] and methanolysed, and the products were examined by g.l.c.

Oligosaccharide *1* (112 mg) had d.p. 2.1, $[\alpha]_D^{18}$ −8° (*c* 1.0, water), R_C values of 1.00, 1.00, and 1.10, and M_{Glc} 0.52. On hydrolysis, the disaccharide gave mannose only. The methylated and methanolysed product had *T* values of 1.19 and 2.10 (peak area 1:1) on phase *G1*, and 1.37 and 4.99 on phase *G2*, corresponding to the values for the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose. On hydrolysis, the methylated disaccharide gave two components having R_{TMG} 1.00 and 0.62, (irrigant *H*), which were indistinguishable from 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose.

Oligosaccharide *2* (69 mg), d.p. 2.9, $[\alpha]_D^{20}$ −23° (*c* 0.5, water), m.p. 168–170°, had R_C 0.50, 0.39, and 0.43, and M_{Glc} 0.52. A hydrolysate contained only mannose. The methylated and methanolysed product had *T* values of 1.19 and 2.08 on phase *G1*, and 1.36 and 4.98 (ratio 2:1) on phase *G2*, corresponding to the methylated sugars isolated from methylated oligosaccharide *1*.

Oligosaccharide *3* (14 mg), d.p. 3.8, had R_C 0.15, 0.12, and 0.12, and M_{Glc} 0.46. Hydrolysates contained only mannose. The methylated and methanolysed product had *T* values of 1.17 and 2.10 on phase *G1*, and 1.38 and 5.01 (ratio 1:3) on phase *G2*, corresponding to the *T* values cited earlier.

Oligosaccharide *4* (12 mg), d.p. 1.95, had R_C 0.75, 0.73, and 0.70, and M_{Glc} 0.41; the R_C values are similar to that of 4-*O*-β-D-mannopyranosyl-D-glucose. A hydrolysate contained glucose and mannose (irrigants *A* and *B*) in the ratio of 1:1. After reduction and hydrolysis, only mannose was detected on paper chromatograms by *p*-anisidine hydrochloride. The methylated and methanolysed product had *T* values of 1.17, 1.52, and 1.81 on phase *G1*, and 1.37, 3.42, and 4.72 on *G2*; the ratio of the first two components was 1:1 on *G2*, and the last two values corresponded to those for methyl 2,3,4,6-tetra-*O*-methyl-α- and β-D-mannopyranosides.

Oligosaccharide *5* (16 mg), d.p. 3.2, had R_C 0.63, 0.40, and 0.48, and M_{Glc} 0.43. Hydrolysis gave glucose and mannose in the ratio 1:1. The methylated and methanol-

ysed product had *T* values of 1.00, 1.17, and 2.09 on phase *G1*, and 1.00, 1.40, and 4.99 on phase *G2*. The first two *T* values on phase *G1* and *G2* corresponded to those given by the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, and the last values to those of 2,3,6-tri-*O*-methyl-D-mannopyranose (ratio 1:2 for parent, methylated sugars).

Oligosaccharide *6* (6 mg), d.p. 2.8, had R_C 0.46, 0.26, and 0.19, and M_{Glc} 0.43. Hydrolysis gave glucose and mannose (paper chromatography) in the the ratio of 1:2. After reduction of *6* and hydrolysis, only mannose was detected on paper chromatograms treated with *p*-anisidine hydrochloride. The trisaccharide, on methylation and methanolysis, yielded products having *T* values of 1.39, 3.44, 4.74, and 4.99 on phase *G2*, compatible with the presence of methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,6-tri-*O*-methyl-D-mannose. The ratios of the three parent, methylated sugars were *ca.* 1:1:1.

Oligosaccharide *7* (9 mg), d.p. 2.9, had R_C 0.30, 0.19, and 0.12, and M_{Glc} 0.46. Hydrolysis gave glucose and mannose (1:2, paper chromatography). After reduction of the oligosaccharide, hydrolysis gave glucose and mannose in the ratio 1:1. After methylation of *7*, methanolysis gave components with *T* values (phase *G2*) of 1.39, 3.45, 1.76, and 5.01. These are compatible with the presence of methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,6-tri-*O*-methyl-D-mannose.

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