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CHEMICAL AND ^{13}C-N.M.R. STUDIES OF AN ARABINOGALACTAN FROM *Larix sibirica L.*

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ABSTRACT

An arabinogalactan isolated from the wood of *Larix sibirica L.* was investigated by methylation analysis, partial hydrolysis, enzymic oxidation, and ^{13}C-n.m.r. spectroscopy. The structural conclusions arrived at by ^{13}C-n.m.r. spectroscopy were consistent with the data from methylation analysis. The polysaccharide is highly branched and similar in structure to those of arabino-3,6-galactans isolated from other *Larix* species.

INTRODUCTION

Arabinogalactans are widely distributed throughout the plant kingdom as hemicellulosic[1] components or constituents of pectic substances of plant cell-walls[2]. They have been found in many plant tissues, cultured cells, and media, and have been isolated as polysaccharides free from, or covalently bound to, proteins[3–5]. The composition, structure, and function of such polysaccharides and glycoproteins have been reviewed briefly[6], and two main structural types have been identified[7].

We now report on the structure of a neutral, water-soluble arabinogalactan isolated from the wood of *Larix sibirica L.* The results of chemical analysis were correlated with those of ^{13}C-n.m.r. spectroscopy.

RESULTS AND DISCUSSION

The cold-water extract of lipid-free sawdust of *Larix sibirica L.* contained polymeric material, $[\alpha]_D$ +10°, which consisted of galactose and arabinose (molar ratio 8.3:1.0). Fractionation of this material by gel-permeation chromatography on Sephadex G-75 gave a polysaccharide, $[\alpha]_D$ +10°, composed of galactose and arabinose in the molar proportions 10:1, which was homogeneous by electrophoresis (12.55×10^{-5} cm^2.V^{-1}.s^{-1}) and sedimentation analysis ($\bar{M}_w$ 2.9×10^4).

Hydrolysis of the arabinogalactan under very mild acidic conditions gave galactose, arabinose, and oligosaccharides **1–6**, which were resolved on Sephadex G-15 and by paper chromatography. They were identified on the basis of methyla-

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tion analysis, mass spectrometry, and ^{13}C-n.m.r. spectroscopy as β-L-Ara*p*-(1→3)-L-Ara (**1**), β-D-Gal*p*-(1→3)-D-Gal*p* (**2**), β-D-Gal*p*-(1→6)-D-Gal*p* (**3**), and β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→3)-β-D-Gal*p* (**4**); **5** comprised two D-galactotetraoses, the structures of which (**5a** and **5b**) were identified on the basis of methylation analysis, and **6** was a D-galactopentaose branched at the reducing unit.

D-Gal*p*-(1→6)-D-Gal*p*-(1→6)-D-Gal*p*
3
↑ **5a**
1
D-Gal*p*

D-Gal*p*-(1→3)-D-Gal*p*-(1→3)-D-Gal*p*
6
↑ **5b**
1
D-Gal*p*

D-Gal*p*-(1→6)-D-Gal*p*-(1→3)-D-Gal*p*-(1→3)-D-Gal*p*
6
↑ **6**
1
D-Gal*p*

The partially methylated saccharides obtained by hydrolysis of the methylated arabinogalactan were identified as the corresponding alditol acetates[8] and aldononitriles[9] by g.l.c. or g.l.c.–m.s.[10]. The results are listed in Table I.

TABLE I

METHYLATED SUGARS FROM THE HYDROLYSATE OF THE METHYLATED ARABINOGALACTAN

Sugar	*Molar proportion*[c]	T[b]		*Linkage indicated*
	I[d]	*Alditol acetates*	*Acetylated aldononitriles*	
2,3,4,6-Me_4-Gal[a]	1.00	1.00	1 00	D-Gal*p*-(1→
2,4,6-Me_3-Gal	0.05	1 47	1.29	→3)-D-Gal*p*-(1→
2,3,4-Me_3-Gal	0.77	1.88	1.38	→6)-D-Gal*p*-(1→
2,4-Me_2-Gal	1.15	2.48	1.76	→3,6)-D-Gal*p*-(1→
2,3,4-Me_3-Ara	0.13	0.61	0.71	L-Ara*p*-(1→
2,5-Me_2-Ara	0.14	0.93	0.94	→3)-L-Ara*f*-(1→

[a]2,3,4,6-Tetra-*O*-methyl-D-galactose, *etc.* [b]G.l.c. on columns *B* and *C*. [c]Values were corrected by use of the effective, carbon-response factors given by Albersheim *et al* [36]. [d]I, Original polysaccharide.

Identification of **1–6** and the results of methylation analysis proved the arabinogalactan macromolecule to have a highly branched structure. The average repeating-unit contained 2 arabinose and 20 galactopyranose residues; 7 galactopyranose residues represented non-reducing ends, 8 residues formed the branches on O-3 and O-6, and 5 residues were (1→6)-linked.

The isolation of 3-*O*-β-L-arabinopyranosyl-L-arabinose (**1**) and the formation of equimolar amounts of 2,5-di-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-L-arabinose on hydrolysis of the methylated polysaccharide indicated that **1** was embodied in the polysaccharide structure through a furanosidic linkage. The occurrence of labile arabinofuranosidic bonds in the polysaccharide explained the absence of oligosaccharides after partial hydrolysis of the polysaccharide. Both (1→3)-linked L-arabino-furanose and -pyranose residues have been found in arabino-3,6-galactans from other *Larix* species, *e.g.*, *L. occidentalis*[11], *L. decidua*[12], and *L. laricina*[13]. However, purified arabino-3,6-galactans from other sources, *e.g.*, cultured cells of sycamore[14], cotton fibres[15], and rape seeds[16] have (1→5)-linked arabinofuranosyl side-chains.

Successive oxidation of arabinogalactan by D-galactose oxidase (ex. *Dactylium dendroides*)[17] and hypoiodite[18,19] afforded a modified acidic polysaccharide, $[\alpha]_D$ −1.5°, containing 24% of uronic acid, in which the terminal D-galactopyranosyl groups had been transformed into β-D-galactopyranosyluronic acid groups. The results of methylation analysis of the original polysaccharide (Table I), which was modified without being markedly degraded, showed that up to 71% of terminal D-galactopyranosyl groups were oxidised. The extent of oxidation was much higher than that (up to 10%) reported for arabinogalactan of western larch[19] and there was considerable decrease in the molecular weight of the latter polysaccharide.

A methanolysate of the methylated modified polysaccharide contained (g.l.c.–m.s.) methylated 6-*O*-(D-galactopyranosyluronic acid)-D-galactose[20], indicating that the terminal galactopyranosyl groups in the side chains were attached exclusively at O-6.

^{13}C-N.m.r. data for **1–3** as well as for the basic sequence (**7**) of the polysaccharide are listed in Table II. The assignment of signals was based on comparison

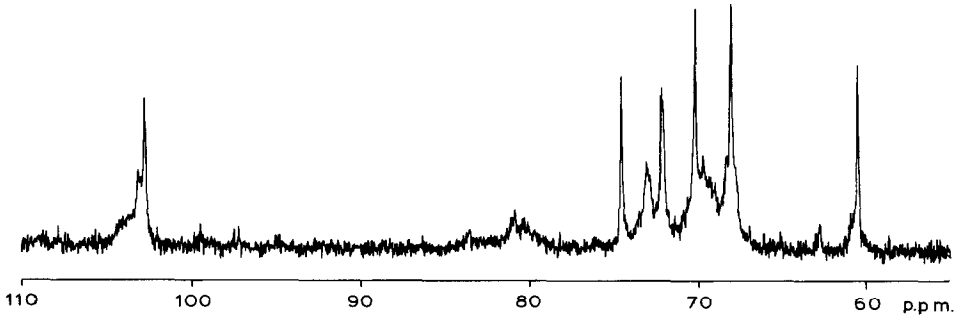

Fig. 1. ^{13}C-N.m.r. spectrum of the arabinogalactan obtained from the wood of *Larix sibirica L.*

TABLE II

^{13}C-N M R DATA FOR *Larix sibirica L.* ARABINOGALACTAN AND THE DERIVED OLIGOSACCHARIDES

Compound	*Residue*	*Chemical shifts (p.p.m.)*					
		C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1 L-Ara*p*-(1→3)-L-Ara	*Aβ*	96.6	69.0	69 3	69.7	63.8	—
A β B	*Bα*	97.4	71.0	78.0	69.7	66.7	—
	Bβ	93.4	69.3	74.5	69.7	62.8	—
2 D-Gal*p*-(1→3)-D-Gal*p*	*Aβ*	103.7	72.3	73.8	70.5	74.8	62.4
A β B	*Bα*	93.5	69.6	79.5	70.0	71.9	62.4
	Bβ	97.7	73.8	80.1	70.0	76.4	62.4
3 D-Gal*p*-(1→6)-D-Gal*p*	*Aβ*	104.0	72.6	73.4	70.1	76.0	61.8
A β B	*Bα*	93.2	69.6	69.9	70.1	71.6	69.6
	Bβ	97.3	73 4	74.6	70.1	74.6	69.1
C							
7 D-Gal*p*-(1→6)-D-Gal*p*-(*1*→6)-D-Gal*p*-(1→	*A(D)*	104.0	73.0	73.9	71.0	75.2	61.3
A β B β 3		(103.6)					
↑ *β*	*B*	104.0	73.0	75.4	71.0	75.4	68.9
1	*C*	103.6	73.9	81 7	70 4	72.9	68.9
D-Gal*p*							
D							

with the data for the respective monosaccharides[21,22] and taking into account the effect of glycosidation. The spectrum of the polysaccharide (see Fig. 1) exhibited signals in the anomeric region at 104.0 and 103.6 p.p.m. attributable both to (1→6)-linked galactopyranosyl residues and units involved in branching through O-3 and O-6. Signals characteristic of C-1 of (1→3)-*β*-linked arabinosyl residues and galactosyl residues were not observed in the spectrum.

The signal for C-6 of galactopyranose at 61.3 p.p.m. and that for C-3 at 73.9 p.p.m. were shifted on glycosidation towards lower field by approximately the same values (7.6 and 7.8 p.p.m., respectively). These chemical shifts are comparable with those in the spectrum of the original polysaccharide and, therefore, the characteristic signals in the spectra of **2** and **3** are useful for structural characterisation of the polysaccharide.

Since the signal intensities were not influenced by either the relaxation processes or n.O.e. under the conditions of spectral measurements of the polysaccharide, they permit conclusions on the relative proportions of the constituent sugars of the polysaccharide which can be compared with the results of the chemical analysis.

Comparison of integrated intensities of the signals for C-6 and C-3 of galactose with those of galactose involved in glycosidic linkages provided important data on the relative proportions of the basic units *A,B* and *B,C* (Table II) in the arabinogalactan. These data indicated that the number of galactose residues in-

volved in glycosidic linkages through O-6 was 2.2 times greater than that of the galactose residues having HO-1 unsubstituted. The ratio of the galactose residues linked through O-3 to free units was 0.77:1.0. These values are comparable to those (1.83 and 0.67) resulting from methylation analysis (Table I). Fig. 1 shows the signals of C-3 of glycosylated galactose to be considerably broadened. Since this phenomenon cannot be explained by changes of T_1 values, it is probably caused by different mobilities of galactosyl residues inside the polysaccharide chain. Broadening of the C-3 signals observed also with 3′-galactosyl-lactose[23] and its homologues depended on the length of the molecule. Thus, broadening of the signals for C-3 in the spectrum of the arabinogalactan can be rationalised by the presence of a chain composed of (1→3)-linked galactopyranosyl residues.

The results of ^{13}C-n.m.r. spectroscopy of the arabinogalactan, together with those of chemical analyses, can be utilised in structure elucidation. The spectroscopy method makes it possible to distinguish the structural heterogeneity of polysaccharides of a given type. Recently, ^{13}C-n.m.r. spectroscopy has been applied in studies of an arabinogalactan from sugar cane[24].

The results indicate a highly branched molecule of arabinogalactan with three different side-branchings (**a–c**) attached to O-6 of (1→3)-linked D-galactopyranosyl residues.

a	**b**	**c**
→3)-β-D-Gal*p*-(1→	→3)-β-D-Gal*p*-(1→	→3)-β-D-Gal*p*-(1→
6	6	6
↑	↑	↑
1	1	1
β-D-Gal*p*	β-D-Gal*p*	Ara*p*-(1→3)-β-L-Ara*f*
6		
↑		
1		
β-D-Gal*p*		

A structure of the arabinogalactan having the average unit **8**, which accords with the methylation analysis, can have various sequences.

→[**a**→**a**→**a**→**b**→**c**→**a**→**a**→**b**]→
8

The arabinogalactan from *Larix sibirica L.* has structural features that are essentially the same as those reported for larch species. Differences are apparent in chemical composition as well as size and shape of the macromolecules associated mostly with the side chains. Similar heterogeneity in branching has also been found with arabinogalactans isolated from field-bean hulls[25].

EXPERIMENTAL

General. — Optical rotations (1-mL cell) were measured at 20 ±1° with a Perkin–Elmer Model 141 polarimeter. Free-boundary electrophoresis of polysaccharide solutions (10 mg/mL) was performed in 0.05M sodium tetraborate buffer (pH 9.2), with a Zeiss 35 apparatus at 150 V and 8 mA for 30 min. G.l.c. was performed on a Hewlett–Packard Model 5711 A instrument, with *A*, a column (305 × 0.3 cm) of 1% of XE-60 on Gas Chrom Z (80–100 mesh) at 130–150° (1°/min), with nitrogen as carrier gas at 36 mL/min; *B*, a column (200 × 0.3 cm) of SP-2340 on Chromosorb WAW-DMCS (80–100 mesh) at 180° (4 min)→210° (2°/min), flow rate 30 mL/min; *C*, a column (200 × 0.5 cm) of OV-225 on Chromosorb WAW-DMCS (80–100 mesh) at 120° (4 min)→180° (2°/min), flow rate 30 mL/min; *D*, a column (220 × 0.2 cm) of SE-30 on Chromosorb WAW-DMCS (100–120 mesh) at 160° (4 min)→280° (4°/min); *E*, a column (400 × 0.4 cm) of 10% of Carbowax 400 on Chromosorb WAW (80–100 mesh) at 45°, flow rate 17 mL/min. Column *A* was used for quantitative analysis of sugar trifluoroacetates[26].

G.l.c.–m.s. was carried out with a JGC-20 K gas chromatograph fitted with column *B* or *C*, 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° and that of the ionising chamber 200°.

P.c. was conducted by the descending method on Whatman No. 1 and 3MM papers with *F*, ethyl acetate–pyridine–water (8:2:1); *G*, ethyl acetate–acetic acid–water (18:7:8); *H*, 1-butanol–ethanol–water–ammonia (4:1:5:trace). Sugars were detected with alkaline silver nitrate and aniline hydrogen phthalate. The mobilities (R_{Gal} and R_G, respectively) are expressed relative to those of D-galactose and 2,3,4,6-tetra-*O*-methyl-D-glucose. The retention times (*T*) of the methylated alditol and aldononitrile acetates are given relative to those of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol and 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactononitrile, respectively. Uronic acids were determined by the modified carbazole method[27], carbohydrates by the phenol–sulphuric acid method[28], and acetyl contents by g.l.c. (β-D-glucose penta-acetate) (column *E*).

^{13}C-F.t.-n.m.r. spectra were measured at 35° for solutions in D_2O (internal MeOH, 50.15 p.p.m. relative to the signal for Me_4Si) with a Bruker WM-250 spectrometer, using the inverse gated-decoupling mode (without n.O.e.); sweep width, 5000 Hz; pulse width, 12 μs; pulse duration, 5 s.

Determination of molecular weight. — A Beckman Model E analytical ultracentrifuge was used. Velocity sedimentation was conducted at 27,500 r.p.m. with a solution of polysaccharide (1 mg/mL). Gel filtration was effected on a column (97 × 1.5 cm) of Sephadex G-75, with Dextran T-10 (mol. wt. 9300), T-20 (mol. wt. 22,300), and T-40 (mol. wt. 39,500) as standards. Water containing 0.02% of sodium azide was used as the eluant.

Composition of the wood. — Standard analytical methods[29] showed that the

larch wood *Larix sibirica L.* (0.2–0.7 mm, extractive-free) contained (%) cellulose (38.0), lignin (23.3), acetyl (1.8), methoxyl (4.2), uronic anhydride (1.9), galactose (15.0), glucose (44.2), mannose (7.4), arabinose (2.1), and xylose (4.1).

Arabinogalactan. — (a) Isolation. Extractive-free wood meal (74.2 g) was extracted twice, with continuous stirring, with water (800 mL) at room temperature for 3 h. The combined extracts were added to ethanol (3 vol.); the precipitate was collected by centrifugation, and washed successively with ethanol and acetone. The light-coloured product (9.6 g, 13.2% based on wood), $[\alpha]_D$ +10° (*c* 1, water), yielded, on hydrolysis, D-galactose and L-arabinose in the molar ratio 8.3:1.0.

A solution of the arabinogalactan (400 mg) in water (2 mL) was applied to a column (2.5 × 100 cm) of Sephadex G-75 (40–120 μm) and irrigated with water. Assay for total carbohydrates (see Fig. 1) revealed two polysaccharides. The appropriate fractions were combined, concentrated to a small volume, and freeze-dried to yield polysaccharides *A* (337 mg, 84.3%), $[\alpha]_D$ +10° (*c* 1, water) (galactose:arabinose 10:1); and *B* (63 mg, 15.7%), $[\alpha]_D$ +12° (*c* 0.8, water) (galactose:arabinose 3:1). Sedimentation analysis ($\bar{M}_w$ 29,000) and electrophoretic mobility (μ 12.55 × $10^{-5}cm^2.V^{-1}.s^{-1}$) confirmed the homogeneity of the polysaccharide *A*, which was used for further investigation.

(b) Partial hydrolysis. The polysaccharide (900 mg) was treated at 100° for 2 h, first with aqueous 10% formic acid (20 mL) and then with aqueous 20% formic acid. The hydrolysate was concentrated after each treatment and the part to be hydrolysed further was precipitated with ethanol. The low-molecular-weight fragments (740 mg), after fractionation on a column (2.5 × 100 cm) of Sephadex G-15 with water, gave D-galactose and a mixture (210 mg) of oligosaccharides. Preparative p.c. on Whatman No. 3MM paper yielded components with R_{Gal} 0.63 (solvent *E*), 0.75, 0.51, 0.28, 0.14, and 0.06 (solvent *F*). The oligosaccharides **1–6** (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 m.s.

Compound **1** (26.8 mg), R_{Gal} 0.63 (solvent *E*), $[\alpha]_D$ +104° (*c* 1, water), gave arabinose on hydrolysis. The intense fragment-ions abJ_1 (*m/z* 235) and aA_1 (*m/z* 175), and ions at *m/z* 115 in the mass spectrum of the fully methylated derivative identified[30] **1** as a (1→3)-linked pentose-disaccharide.

Compound **2** (19.4 mg), R_{Gal} 0.75 (solvent *F*), $[\alpha]_D$ +42° (*c* 0.8, water), gave galactose on hydrolysis. The mass spectrum of methylated **2** contained characteristic peaks of ions baJ_1 (*m/z* 279) and aA_1 (*m/z* 219), and ions at *m/z* 159. The calculated mol. wt. (454) and the intense ions at *m/z* 159[20], respectively, confirmed the (1→3)-linked hexose-disaccharide structure.

Compound **3** (68 mg), R_{Gal} 0.51 (solvent *F*), $[\alpha]_D$ +24° (*c* 2, water). The mass spectrum of methylated **3** contained ions characteristic for **2** (*m/z* 279, 219), and also an intense ion of the fragment abD_1 (*m/z* 353), which proved the (1→6)-linked disaccharide structure.

Compound **4** (16 mg), R_{Gal} 0.28 (solvent *F*), $[\alpha]_D$ +18° (*c* 0.6, water), gave galactose on hydrolysis. The abundant ions at *m/z* 159 in the mass spectrum, to-

gether with the calculated mol. wt. of 658, proved the presence of the (1→3) linkage in the methylated hexose-containing trisaccharide[20,31]. The mass spectrum of the methylated trisaccharide-alditol ($NaBD_4$) contained intense peaks for fragments H_1 (*m/z* 88), bcA_1 (*m/z* 440), and bcJ_1 (*m/z* 236)[32] which characterise the (1→6) linkage between units *a* and *b*. The methylated trisaccharide-alditol was hydrolysed with 0.1M hydrochloric acid, the hydrolysate was neutralised, and the resulting sugars were converted into alditol acetates. G.l.c.–m.s. then identified 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl-D-galactitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol, and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-galactitol. Thus, **4** is *O*-D-galactopyranosyl-(1→6)-*O*-D-galactopyranosyl-(1→3)-D-galactose.

Compound **5** (26.6 mg), R_{Gal} 0.14 (solvent *F*), $[\alpha]_D$ +17° (*c* 1.1, water), gave galactose on hydrolysis. The mass spectrum of methylated **5** contained intense ions of the A series ($cabA_1$ *m/z* 627, baA_1 423, aA_1 219), suited for calculation of the mol. wt. Thus, the mol. wt. of 862 proves **5** to be a hexose-containing tetrasaccharide[33]. Methylated **5** was hydrolysed and the sugars obtained were analysed by g.l.c.–m.s. as the alditol acetates; 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-galactitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-galactitol, and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-D-galactitol were identified. These findings, together with the fact that the mass spectrum of methylated **5** contained all the peaks of ions J shifted by 60 mass units (*m/z* 687, 483, and 279), corresponding to those of ions A, provide evidence[33] that **5** consisted of at least two tetrasaccharides having (1→3) and (1→6) linkages, the branching point being at O-3 and O-6.

Compound **6** (24.2 mg), R_{Gal} 0.06 (solvent *F*), $[\alpha]_D$ +15° (*c* 1, water), gave galactose on hydrolysis. The mass spectrum of **6** contained, in comparison with that of **5**, a further peak of series A, namely $dabcA_1$, at *m/z* 831. The calculated mol. wt. of 1066 indicated a hexose-pentasaccharide molecule. G.l.c.–m.s. of the alditol acetates, obtained after hydrolysis of methylated **6**, showed the same components as from **5** with an increased proportion of 2,4,6-tri-*O*-methyl-D-galactose. The oligosaccharide contained (1→3) and (1→6) linkages, and the very weak peak for the deJ_1 ion at *m/z* 279 indicated the branch point in **6** to be almost exclusively in the fourth (*d*) unit[33].

(c) Methylation analysis. The polysaccharide (55 mg) was methylated once by the Hakomori method[34] and twice by the Purdie method[35] to give a product (53 mg), $[\alpha]_D$ −51° (*c* 1, chloroform) (Found: OMe, 45.5%). The methylated polysaccharide (10 mg) was treated with aqueous 90% formic acid (2 mL) at 100° for 1 h, the hydrolysate was concentrated to dryness, and the residue was hydrolysed with 2M hydrochloric acid (2 mL) at 100° for 6 h. P.c. then revealed di-, tri-, and tetra-*O*-methyl saccharides with R_G 0.47, 0.54, 0.76, 0.85, and 0.93 (solvent *H*), which were converted into their alditol acetates and aldononitrile acetates[8,9] and analyzed by g.l.c.–m.s.[10] (columns *B* and *C*). The methylated sugars detected are listed in Table I.

(d) Oxidation of the terminal D-*galactopyranosyl groups.* A solution of the

polysaccharide (88 mg) in 0.02M sodium phosphate buffer (pH 7.0, 5 mL) was treated under toluene with D-galactose oxidase (D-galactose:O_2 oxidoreductase, EC 1.1.3.9) from *Dactylium dendroides* (specific activity 4.16 kat.kg^{-1})[17] (3.334 μkat) and catalase (Reanal, Budapest) (340 μkat)[18,19]. After 65 h at ambient temperature, iodine (250 mg) and a solution of sodium carbonate (330 mg) in water (55 mL) were added. The mixture was stirred for 2 h and then extracted (60 min with stirring) with an equal volume of aqueous 90% phenol. After centrifugation, the aqueous layer was removed and dialysed (48 h) against water. The oxidised polysaccharide (77.8 mg, 92%), $[\alpha]_D$ −1.5° (*c* 1, water), was recovered by freeze-drying (Found: uronic acid, 24%).

The oxidised polysaccharide (30 mg) was methylated by the Hakomori[10] procedure; the product had no i.r. hydroxyl absorption. A sample (5 mg) of the product was treated with anhydrous methanolic 3% HCl for 12 h at 100° and the products were analysed by g.l.c.–m.s. (column *D*). The mass spectrum of the methylated disaccharide (*T* 9.57) (calculated mol. wt., 454) contained characteristic ions baD_1, abJ_1, aA_1, bA_1 at *m/z* 367, 279, 233, and 219, respectively, which confirmed[20] the linkage to be (1→6).

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