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Note

Isolation and structure of a 4-*O*-methyl-glucuronoarabinogalactan from *Boswellia serrata*

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The plant species *Boswellia serrata* Roxb belongs to the genus *Boswellia* Roxb (*Burseraceae*) and is well known for its medicinal uses[1]. The tree, on tapping, exudes an oleo-gum resin (Salai gum guggul, Frankmanse) that is reported to have anti-inflamatory, anti-arthritic, and strong hypolipidaemic drug activities. Few chemical studies have been undertaken on the polysaccharides of this plant species. The present communication is concerned with the isolation and structural elucidation of a 4-*O*-methyl-glucuronoarabinogalactan from this resin.

RESULTS AND DISCUSSION

The water-soluble portion of the defatted plant material was fractionated by repeated precipitation with ethanol. Hydrolysis of the crude polysaccharide gave two neutral sugars, arabinose and galactose, together with a hexuronic acid. Ion-exchange chromatography gave five different fractions, the major one of which on gel-permeation chromatography afforded a homogenous, N-free polysaccharide. The polysaccharide was electrophoretically homogenous and had mol. wt. 5.1–5.6 × 10^5 against dextran standards[2]. The polysaccharide had $[\alpha]_D$ − 10.5° and contained 69% neutral sugars by the phenol–H_2SO_4 method[3] and 64% by the anthrone method[4]. Prolonged hydrolysis gave D-glucuronic acid, as detected by p.c. and estimated by a modified carbozole method[5] as 18.2% for native and 20.4% for the mild acid-degraded polysaccharide.

The presence of GlcA was also confirmed by strong C-6 signals at δ 176.5 and 175.2 in ^{13}C-n.m.r. of both the native and degraded polysaccharides[6–11]. A signal at δ 103.7 may be attributed to C-1 of β-linked 4-*O*-methyl-D-glucuronic acid, the methyl

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group of which resonated at δ 60.74. Signals at δ 109.9 and 108.3 could be attributed to C-1 of the terminal and internal L-arabinofuranose residues as mild hydrolysis of the polysaccharide led to loss of these signals. From DEPT sequence-studies it was evident that a signal at δ 60.74 is that of a primary carbon atom, $-CH_3$, whereas a signal at δ 61.7, which had a negative sign, was assigned to a the secondary carbon atom, $-CH_2$ that is, C-6 of galactopyranose. Strong downfield signals[12] at δ 68.9 and δ 70.0 in the spectrum of the native polysaccharide were assigned to substituted C-6 of galactopyranosyl residues, and a signal at δ 61.7 for unsubstituted C-6 suggested a high proportion of (1→6)-β-D-galactan branches, whereas a strong downfield signal at δ 82.8 was assigned to C-3 of the (1→3)-β-D-galactan core. After mild acid hydrolysis, partial enhancement of the uronic acid signals occurs with complete loss of arabinose side-chains, indicating that 4-*O*-methyl-glucuronic acid is linked exclusively to core β-D-galactose residues and not to be L-arabinose residues. A weak signal at high field region (δ 17.3) is attributable to a traces of rhamnosyl residues.

Hydrolysis of the permethylated[13,14] polysaccharide and quantitation of the partially methylated sugars as their alditol acetates (Table I) yielded 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-galactose, 2,3,5-tri-*O*-methyl-L-arabinose, 2,4-di-*O*-methyl-D-galactose, and 2,5-di-*O*-methyl-L-arabinose. The formation of 2,3,5-tri-*O*-methyl-L-arabinose indicates that the most of the L-arabinose residues are present as terminal non-reducing furanosyl groups, and the formation of 2,3,4,6-tetra-*O*-methyl-D-galactose shows that a few D-galactose residues are also present as non-reducing terminal pyranosyl groups. The formation of high proportions of 2,3,4- and 2,4,6-tri-*O*-methyl- and 2,4-di-*O*-methyl-D-galactose suggests

TABLE I

Methylation analysis of a, native polysaccharide; b, carboxyl-reduced polysaccharide; c, acid-degraded polysaccharide; d, saccharides **1**, and **2** and carboxyl-reduced saccharides **3**, and **4**

Methylated sugar[a]	*Retention time T*[b]		*Mol. proportions*[c]							*Deduced linkages*
			A	*B*	*C*	*D*				
	(i)	*(ii)*				*1*	*2*	*3*	*4*	
2,3,4,6-Gal	1.22	1.21	12.2	9.9	14.2	1	0.9			T Gal*p*-(1-
2,3,4,6-Glc[d]	1.10	1.04		15.3				1.0	0.9	T Glc*p*-(1-
2,3,4-Glc[d]	2.42	2.18	2.1		1.7					T Glc*p*A-(1-
2,3,4-Gal	3.38	2.84	24.2	20.2	27.9		1.0	1.1	1.9	-6)-Gal*p*-(1-
2,4,6-Gal	2.31	2.11	11.9	10.9	14.1	1.1				-3)-Gal*p*-(1-
2,3,5-Ara	0.42	0.47	6.3	5.2						T Ara*f*-(1-
2,4-Gal	6.28	5.16	36.5	30.1	42.1					-3,6)-Gal*p*-(1-
2,5-Ara	1.05	0.81	5.9	5.6						-3)-Ara*f*-(1-

[a] 2,3,4,6-Gal = 2,3,4,6-tetra-*O*-methyl-D-galactose, etc. [b] Retention times for the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-glucitol on (*i*) a 3% ECNSS-M column (*ii*) a 3% OV-225 column, both at 170°. [c] Average value. [d] Derived from 4-*O*-methyl-D-glucuronic acid residues.

the presence of (1→3)-linked and (1→6)-linked galactan chains, being either main chain or branching with frequent substitution at O-6 and O-3, respectively. The small amount of 2,3,4-tri-*O*-methyl-D-glucose was derived from 4-*O*-methyl-D-glucuronic acid.

On periodate oxidation, the native polysaccharide consumed 0.82 mol of periodate per hexosyl residue. This value is in good agreement with the theoretical value of 0.88 calculated on the basis of the methylation data. The results of methylation analysis of the carboxyl-reduced[15] polysaccharide indicates that all the 4-*O*-methyl-glucoronic acid residues were at the non-reducing ends of the (1→6)-β-D-galactopyranosyl chains. Methylation studies of the polysaccharide degraded[16] by mild acid also suggests that the labile (1→3)-α-L-arabinofuranosyl side-chains are linked to (1→6)-β-D-galactopyranosyl residues and the 4-*O*-methyl-D-glucuronic acid residues are located on the galactan chain.

The periodate-oxidized[17], Smith[18]-degraded native polysaccharide gave a non-dialysable polysaccharide fraction, hydrolysis of which showed the presence of D-galactose only, and methylation analysis confirmed the presence of a (1→3)-β-D-galactan core in the native polysaccharide.

Graded hydrolysis[19] of the polysaccharide afforded two homogenous, neutral oligosaccharides (**1** and **2**) and two homogenous acidic oligosaccharides (**3** and **4**).

Saccharides **1** and **2** (R_{Gal} 0.54 and 0.35 respectively, solvent *C*) on acid hydrolysis afforded only D-galactose. Methylation analysis yielded 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,4,6-tri-*O*-methyl-D-galactose in equal proportions for **1** and 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4-tri-*O*-methyl-D-galactose in equal proportions for **2**. The $[\alpha]_D$ values (+48.7° for **1** and +43.5° for **2**) were in good agreement with the literature values[20] (+50° and +45°) suggesting β-linkages for both saccharides.

Saccharides **3** and **4** (R_{Gal} 0.58 and 0.46 respectively, solvent *D*) on acid hydrolysis gave 4-*O*-methyl-D-glucuronic acid (R_{Gal} 2.58, solvent *D*) and D-galactose. Treatment of **3** and **4** with methanolic 2% hydrogen chloride followed by sodium borohydride, and subsequent acid hydrolysis of the products gave D-galactose and 4-*O*-methyl-D-glucose in 1:1 and 2:1 proportions, respectively. Methylation analysis of the methyl glycoside of the carboxyl-reduced oligosaccharides **3** and **4** yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-galactose in equal proportions for **3** and 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-galactose in 1:2 proportions for **4**. The $[\alpha]_D$ value (+1.5°) of **3** was in good agreement with the literature[21] value (+1.0)°, suggesting the linkage to be β. Saccharides **1–4** were thus identified as shown.

β-D-Gal*p*-(1→3)-D-Gal*p*

1

β-D-Gal*p*-(1→6)-Gal*p*

2

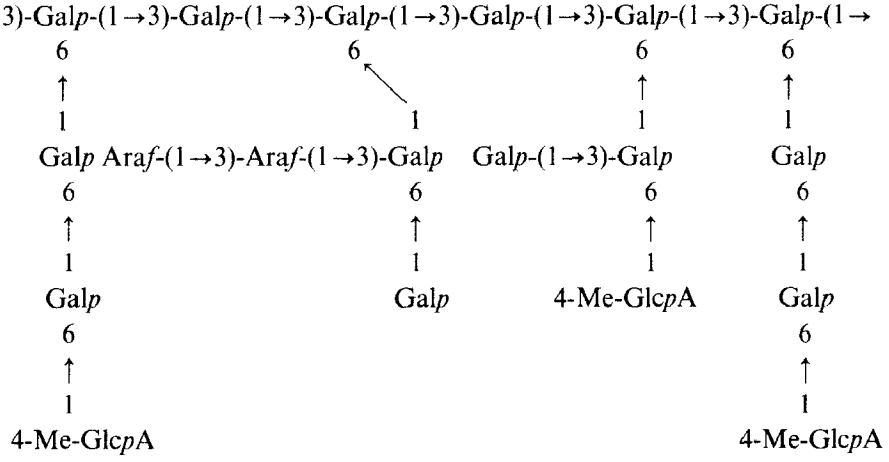
4-Me-β-D-Glc*p*A-(1→6)-D-Gal*p*

3

4-Me-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→6)-D-Gal*p*

4

The foregoing results and literature data indicate that the gum possesses an internal (1→3)-β-D-galactan core with an appreciable proportion of (1→6)-β-D-galactopyranosyl branches, along with sidechains of (1→3)-α-L-arabinofuranosyl subunits and also terminal, non-reducing 4-*O*-methyl-D-glucopyranosyluronic acid residues linked to the galactan branches. Structure **5** is thus tentatively proposed for the Salai guggul polysaccharide, although it does not necessarily depict the correct sequence of branches.



As this type of subunit is still a matter of speculation for most of the plant arabinogalactans, it is likely that they probably do not have any strictly regular repeating-units; however **5** may constitute one main subunit of this highly branched macromolecule.

EXPERIMENTAL

Materials. — The exudate oleo-gum resin of *Boswellia serrata* (*Bureseraceae*) was a gift from Dr. Y. K. Sarin, RRL, Jammu, India. DEAE-Sephacel, Sepharose CL-4B, and Sephadex were purchased from Sigma, U.S.A., and Bio-gel P-2 (200–400 mesh) from Bio-Rad.

General methods. — Component sugars were determined by p.c. of the acid hydrolysate (2M CF_3CO_2H, 2 h, 120°) using Whatman 1 MM paper in the following solvent systems: *A*, 6:4:3 BuOH–C_5H_5N–H_2O; *B*, 4:1:5 BuOH–EtOH–H_2O; *C*, 10:4:3

EtOAc–C_5H_5N–H_2O; *D*, 18:3:1:4 EtOAc–HOAc–HCO_2H–H_2O; and sugars were detected by using alkaline $AgNO_3$. Optical rotations were measured with a Jasco DIP-360 polarimeter at 25°. G.l.c. (Hewlett–Packard 5730A) was used flame-ionisation and thermal conductivity detection (dual port). ^{13}C-n.m.r. spectra were determined by a 300-MHz n.m.r. spectrometer with D_2O as the internal-lock signal. Chemical shifts for ^{13}C are given in δ values relative to the internal standard acetone (31.07 p.p.m.). H.p.l.c. was conducted with an LKB Model 2150 dual-piston pump equipped with a 2238 Uvicord S-II detector and a Knauer differential refractometer. Total carbohydrate was determined by the PhOH–H_2SO_4 and anthrone methods with galactose as the standard. Acid sugars were estimated by modified carbazole method.

Isolation and purification. — The defatted dried powder of the golden yellow "crystals" (10 g) of Salai oleo-gum resin was extracted with H_2O for 15 h at room temperature. The extract was centrifuged (8000 r.p.m.) and the supernatant dialysed, concentrated (250 mL), and the polysaccharide precipitated. It was redissolved and reprecipitated (EtOH) twice more to afford a yellowish-white power (2.4 g). This purified SL gum polysaccharide (500 mg) was applied to a column (80 × 2.6 cm) of DEAE-Sephacel (Cl^- form). The main fraction, eluted with 0.25M NaCl was collected, dialysed for 72 h, and then lyophilized. A white fluffy solid was obtained; yield 43%. The sugar constituents were analysed by g.l.c. (Gal 84%, Ara 11.5%). The foregoing polysaccharide was applied to a column (78 × 2.6 cm) of Sephadex G-100 eluted with 20mM aqueous NaN_3. The main fraction (85–120 mL) was SL 0.25; yield 89%.

Determination of homogeneity and molecular weight. — Homogeneity was proved by high-pressure l.c. using Ultropac TSK G 5000 PW columns (0.4 × 50 cm), gel filtration on a column of Sephadex G-100, and by DISC-PAGE using 7.7% polyacrylamide separating gel and 4.3% stacking gel. The M_r calibration-curves were performed with dextran T 2000, T 500, T 70, T 40, and T 10; a 20mM solution of NaN_3 was used as eluent for Sepharose CL-4B (70 × 1.6 cm).

Hydrolysis of the polysaccharide. — (a) Polysaccharides/oligosaccharides (1 mg) were hydrolysed with 2M CF_3CO_2H (1 mL) for 2 and 1 h respectively at 120° in a sealed tube and the products reduced to the corresponding alditols, which were then acetylated for analysis by g.l.c. on 3% ECNSS-M and 3% OV-225 columns.

(b) Polysaccharide (10 mg) was hydrolysed with 0.1M CF_3CO_2H (5 mL) for 20 min at 95° to afford the mild acid-hydrolysed product.

(c) Crude polysaccharide (600 mg) hydrolysed with 0.5M CF_3CO_2H (50 mL) for 1.5 h at 100°. The neutral and acidic oligomers were separated on a column of Dowex (OAc^-) ion-exchange resin and a concentrated solution of them was applied to a column (1.6 × 90 cm) of Bio-gel P-2 separately, and all the four oligomers were isolated pure. These oligosaccharides were finally purified by preparative p.c. on Whatman 3 MM paper (Solvent system *D*, 40 h).

(d) Oligosaccharides/reduced oligosaccharides (1 mg) were hydrolysed with 2M CF_3CO_2H (5 mL) for 1 h at 121° in a sealed tube and the products isolated conventionally.

Reduction analysis. — (a) Polysaccharide (10 mg) was reduced with 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide-metho-*p*-toluenesulphonate (CMC, 200

mg) at pH 4.75 and the product, after hydrolysis and conversion into alditol acetates was analysed by g.l.c. on a 3% ECNSSM column.

(b) Methylated polysaccharide (5 mg) was methanolised with M methanolic HCl for 12 h under reflux and then reduced with $NaBH_4$ (25 mg). The hydrolysed product was then analysed by g.l.c. as the alditol acetates.

(c) Acidic oligosaccharides (2 mg) each were also esterified with M methanolic HCl for 6 h at reflux, reduced with aqueous $NaBH_4$, and the products identified by g.l.c. of alditol acetates from the hydrolysate.

Methylation analysis. — (a) Polysaccharide/reduced polysaccharide (5 mg) dissolved in dry Me_2SO (5 mL) was methylated with methylsulphinyl carbanion (2.5 mL) and MeI (2.5 mL) according to the method of Hakomori followed by the Purdie method.

(b) Neutral oligosaccharides/reduced oligosaccharides (2 mg) dissolved in dry Me_2SO (2 mL) were methylated by the Hakomori method and treated as described earlier.

Periodate oxidation and degradation. — Polysaccharide (25 mg) was oxidised with 0.1M $NaIO_4$ (45 mL) at 4° in the dark for 72 h and the non-hydrolysable fraction was reduced with $NaBH_4$ (10 mg) for 24 h at room temperature. One part of the non-dialysable fraction (1 mg) was analysed by g.l.c. after hydrolysis and alditol acetylation. The other part (3 mg) of it was methylated by the Hakomori method, hydrolysed and analysed as the partially methylated alditol acetates by g.l.c. as described earlier.

N.m.r. studies. — ^{13}C-N.m.r. spectra of the native, degraded, and modified polysaccharides, and neutral and acidic oligosaccharides were obtained for 2% solutions in D_2O at 75.468 MHz and at 80° (353°K); chemical shifts are expressed relative to that of the internal standard Me_2CO (δ 31.7) with respect to Me_4Si as 0.0).

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