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Note

Structural studies of a polysaccharide from the seeds of *Salmalia malabarica*

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The polysaccharides isolated from the defatted seeds of *Salmalia malabarica* (Bombacaceae) were fractionated into Fractions I (48%), II (30%), III (14%), and IV (8%) using a column of Sephadex G-100 in pyridine–acetate buffer. The principal polysaccharide fraction, Fraction I (carbohydrate content[1] 98%), was found to be electrophoretically homogeneous. Hydrolysis (M sulfuric acid, 16 h, 100°) of Fraction I revealed galactose and arabinose (Table I) by paper chromatography in a relative ratio of 7:3 as determined by gas-liquid chromatography (g.l.c.) of their alditol acetates[2] using *myo*-inositol as the internal standard. The configuration of the galactose and arabinose were determined as D and L, respectively, from their optical rotation measurements (see Experimental section). Complete methylation of Fraction I by the Hakomori method[3] and hydrolysis of the methylated polysaccharide yielded 2,3,5-tri-*O*-methyl-L-arabinofuranose (4.1 mol. equiv.), 2,3,4-tri-*O*-methyl-L-arabinopyranose (11 mol. equiv.), 2,3,4,6-tetra-*O*-methyl-D-galactose (0.85 mol. equiv.), 2,3,6-tri-*O*-methyl-D-galactopyranose (10.8 mol. equiv.), 2,4,6-tri-*O*-methyl-D-galactopyranose (2.2 mol. equiv.), 3,6-di-*O*-methyl-D-galactose (12.4 mol. equiv.), and 3,4-di-*O*-methyl-D-galactose (7.0 mol. equiv.) (see Table II). These products reveal that both L-arabinopyranose and L-arabinofuranose are present at the non-reducing ends. A small number of the non-reducing termini are occupied by D-galactose. Appearance of a large molar proportion of the tri-*O*-methyl pentoses shows that the polysaccharide is highly branched. The major portion of the interior part consists of D-galactose residues linked (1→2,6) and (1→2,4). A portion of the chain was made by D-galactose linked principally 1→4. There was a small portion of D-galactose residues linked (1→3). The polysaccharide Fraction I consumed about 0.9 mol. equiv. of periodate per mole of hexosyl residue as was monitored spectrophotometrically[4,5]. Only D-galactose was detected in the paper chromatogram when the periodate-oxidised, borohydride-reduced material was hydrolyzed with sulfuric acid (0.5M). This observation was in fair agreement with the linkage pattern as suggested from the methylation analysis.

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EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer Model 241 MC spectropolarimeter at 589.6 nm. I.r. spectra were recorded with a Beckman Acculab-10 infrared spectrophotometer using KBr discs. The spectrophotometric readings were taken from a Beckman Model 26 spectrophotometer. Evaporation of water and other solvents was achieved under diminished pressure at a bath temperature ~45°. Paper chromatography (descending) for detection and preparative isolation of the sugars was performed on Whatman No. 1 and Whatman No. 3MM paper, respectively, using solvent mixtures (v/v) (*A*) 8:3:1 ethyl acetate–pyridine–water and (*B*) the upper layer of 4:1:5 butanol–acetic acid–water, and (*C*) the upper layer of 4:1:5 butanol–ethanol–water. Visualization reagents were (a) ammoniacal silver nitrate[6] and (b) saturated aqueous aniline hydrogen oxalate[7]. Gel filtration elution was monitored spectrophotometrically with a Beckman Model 26 spectrophotometer. The g.l.c. of the simple alditol acetates[2] and partially methylated alditol acetates[8] was performed in a Packard–Becker 419 gas chromatograph using either of two columns: Column 1, 3% ECNSS-M on Gas Chrom-Q (100–120 mesh), and Column 2, 3% OV-225 on Gas Chrom-Q (100–120 mesh).

Homogeneity of the polysaccharide by high-voltage paper electrophoresis studies. — High-voltage paper electrophoresis was carried out with a Shandon Model L-24 instrument, using Whatman No. 1 paper, an applied voltage of 600 V and 15 mA, and borate buffer pH 10.4 at a temperature of 2°. In one experiment, with visualization of the zones with benzidine–periodate reagent[9], a single spot was revealed. In another experiment, with segmental analysis according to the method described by Northcote[10], a single zone was again detected.

Isolation and purification of the polysaccharide. — Air-dried seeds (500 g) of *S. malabarica* were extracted successively with petroleum ether (b.p. 60–80°) and methanol in Soxhlet extractors. The extracted seeds were stirred with cold water (2 L) in a mechanical stirrer for 24 h and centrifuged at 6000*g*. The centrifugate was diluted with ethanol (5 L), whereby a silky white precipitate appeared. This mass was again dissolved in water and reprecipitated (yield ~15 g). A portion (0.25 g) of the polysaccharide was purified through fractionation over a column of Sephadex G-100 (2.5 × 100 cm) using pyridine–acetate buffer (pH 4.5) as the eluent. The principal polysaccharide fraction (Fraction I) that appeared at the end of the void volume was collected, dialyzed against distilled water, and lyophilized (yield 120 mg). The polysaccharide, $[\alpha]_D^{22}$ +18° (*c* 0.25, 0.1M NaOH) was shown to be free from uronic acid[11], and also free from halogen, nitrogen, and sulfur, as found from elemental analysis[12].

Acid hydrolysis of the polysaccharide. — Purified polysaccharide (50 mg) was hydrolyzed with M sulfuric acid (25 mL) for 16 h at 100°, using *myo*-inositol (5 mg) as the internal standard. The solution was neutralized with $BaCO_3$, decationized with Amberlite IR-120 [H^+] resin, and concentrated in a rotary evaporator at ~45°. Paper chromatography (solvents *A* and *B*) of the hydrolyzate revealed arabinose and galactose (Table I), the identities of which were further confirmed by g.l.c. (Column 1) of the

TABLE I

Products of acid hydrolysis of the polysaccharide[a]

Sugars	$[\alpha]_D^{22}$ *(water)*	R_{Gal}[b]		T[c]	*Relative ratio*
		Solvent A	*Solvent B*		
D-Galactose	+80° (lit.[d] +79°)	1.0	1.0	0.87	7
L-Arabinose	+103° (lit.[e] +105°)	1.58	1.6	0.28	3

[a] See Experimental for details. [b] R_F values of the sugars, relative to that of D-galactose as unity. [c] Retention times of the corresponding alditol acetates, relative to that of 1,2,3,4,5,6-hexa-*O*-acetyl-D-glucitol as unity. [d,e] Literature values were taken from refs. 13 and 14, respectively.

derived alditol acetates[2]. Their complete identification as D-galactose and L-arabinose was then achieved by isolation through preparative p.c. (solvent *C*), followed by optical rotation measurements. These results are incorporated into Table I.

Methylation analysis. — The polysaccharide (30 mg) was subjected to two successive Hakomori methylation reactions[3] using Me_2SO (15 mL) and methylsulphinyl sodium (15 mL, 30 mmol) to yield the fully methylated polysaccharide (34 mg), which had no signal for hydroxyl groups in the infrared spectrum. The fully methylated polysaccharide was heated under reflux with 90% formic acid (15 mL), and after evaporation of the formic acid, followed by co-distillation with water under reduced pressure, the residue was hydrolyzed with 0.5M sulfuric acid for 12 h at 100°. The hydrolyzate, after neutralization with $BaCO_3$, was decationized with Amberlite IR-120 [H^+] resin and evaporated *in vacuo*. The residue was repeatedly extracted with hot chloroform (4 × 5 mL), and the combined extracts were evaporated to dryness,

TABLE II

Results of methylation analysis of the polysaccharide[a]

Methylated sugars[b]	T[c]		*Molar proportion*	*Linkage pattern*
	Col. 1	Col. 2		
2,3,4,6-Gal	1.25	1.19	0.85	Gal*p*-(1→
2,3,5-Ara	0.48	0.41	4.10	Ara*f*-(1→
2,3,4-Ara	0.73	0.54	11.00	Ara*p*-(1→
2,3,6-Gal	2.42	2.22	10.80	→4)-Gal*p*-(1→
2,4,6-Gal	2.28	2.03	2.20	→3)-Gal*p*-(1→
3,6-Gal	4.35	—	12.40	→2,4)-Gal*p*-(1→
3,4-Gal	6.93	5.5	7.00	→2,6)-Gal*p*-(1→

[a] See Experimental for details. [b] 2,3,4,6-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol, etc. [c] Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity, on a column of 3% ECNSS-M (Col. 1) and 3% OV-225 (Col. 2).

redissolved in water (5 mL), and then reduced with sodium borohydride (3.12 mmol). After neutralisation of the borohydride with acetic acid (0.4 mL), it was decationized with Amberlite IR-120 [H^+], freed from boric acid by repeated co-evaporation with dry methanol, and finally dried over P_2O_5 at 40° *in vacuo*. The product was then acetylated with 1:1 acetic anhydride–pyridine (5 mL, 26.5 mmol) for 25 min at 100°. After repeated evaporation of the solvents with dry toluene (4 × 5 mL), the product was extracted with 10:1 chloroform–methanol (5 mL), and analyzed by g.l.c.[8] For experimental results, see Table II.

Periodate oxidation of the polysaccharide. — The polysaccharide (25 mg) was dissolved in water (100 mL) and treated with 0.5M sodium metaperiodate (20 mL) at 15° in the dark. Consumption of periodate, monitored spectrophotometrically at 225 nm, became constant after 30 h. The excess of metaperiodate was decomposed with ethylene glycol, and the solution was dialyzed. The dialyzed solution was concentrated, and it was then reduced with sodium borohydride (6.6 mmol) and left standing overnight at room temperature. Excess borohydride was decomposed by addition of acetic acid (0.5 mL), and the solution was decationized with Amberlite IR-120 [H^+] resin and evaporated to dryness. The free boric acid was removed by repeated co-evaporation with methanol, and the residue was hydrolyzed with 0.5M sulfuric acid (10 mL) for 8 h at 100°. After usual processing, the hydrolyzate was concentrated *in vacuo* at 45°. Paper chromatography of the hydrolyzate using solvent *A* revealed the presence of traces of galactose as the only monosaccharide which survived the oxidation–hydrolysis procedure.

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