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Note

Structural studies of the D-galacto-D-mannan from the seeds of *Parkinsonia aculeata* Linn.

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Different parts of the *Parkinsonia aculeata* Linn. plant investigated[1–4] have wide industrial and medicinal importance. We report herein on the galactomannan from the seeds of *P. aculeata*.

The water-soluble polysaccharide was isolated by ethanol precipitation, and complexation with Fehling solution, from an aqueous extract of the crushed and defatted seeds of *P. aculeata*. Its homogeneity was established by paper electrophoresis[5], free-boundary electrophoresis[6], and ultracentrifugal analysis[7,8]. The granular, white polysaccharide was nonreducing, free from nitrogen, methoxyl, pentose residues, starch, and uronic acid, had a weight-average molecular weight of ~111,000 (by ultracentrifugation[9]), and yielded D-galactose and D-mannose in the molar ratio of 5:9 on acid hydrolysis (see Table I). I.r. absorption bands at 875 and 820 cm^{-1} suggested the presence of α and β linkages. Partial hydrolysis released, first, D-galactose, followed by D-mannose and five oligosaccharides (1–5). On hydrolysis oligosaccharides **1**,**2** and **4** yielded monosaccharides only, whereas oligosaccharide **5** was cleaved into D-mannose and disaccharide **3** (see Table II). The galactomannan was completely methylated by the Haworth[10] and Hakomori[11] methods, and the product hydrolyzed. By p.c. were observed six methylated neutral monosaccharides whose identities were confirmed by g.l.c. of their alditol acetate derivatives[12] (see Table III). The methylation results demonstrated a branched galactomannan with the sugars in the pyranose form. The polysaccharide consumed 1.05 mol of periodate per mole of glycose residue, with simultaneous liberation of 0.28 mol of formic acid. Subsequent reduction and complete acid hydrolysis gave galactose, mannose, erythritol, and glycerol in the molar ratios of 2:1:7:4.

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TABLE I

CHARACTERIZATION OF THE NEUTRAL SUGARS OBTAINED FROM *P. aculeata* GALACTOMANNAN

Monosaccharides	*ET*[a]	R_t[b]	*Molar ratio*		*Derivatives*[c]
			h.p.l.c.	*g.l.c.*	*m.p. & m.m.p.*
D-Galactose	8.3	21.0	1	1	213–214
D-Mannose	7.1	17.4	1.83	1.85	216–219

[a]Elution time (minutes) of neutral sugars on h.p.l.c. analysis; column, μBondapak/carbohydrate; mobile phase,3:17 (v/v) H_2O–MeCN. [b] Retention time (minutes) of alditol acetates of neutral sugars in g.l.c. analysis; column,ECNSS-M, 3%. [c] *N-p*-Nitrophenyl-D-glycosylamine derivative; the m.p. and m.m.p are in conformity with literature data[22].

The foregoing results showed that the galactomannan is multi-branched, with (1→3), (1→4), and (1→6) linkages.

EXPERIMENTAL

General methods.—All evaporations were performed under diminished pressure at 40–50°. I.r. spectra were recorded with a Perkin-Elmer model 457A spectrophotometer. Descending paper chromatography (p.c.) was performed with the following solvent systems (v/v):(*A*) 4:1:5 BuOH–EtOH–H_2O, (*B*) 3:1:3 EtOAc–AcOH–H_2O, (*C*) butanone–water azeotrope, and (*D*) 4:3:2:2 EtOAc–AcOH–BuOH–H_2O. The detection reagents used were: (*a*) $AgNO_3$ in acetone–alcoholic sodium hydroxide, (*b*) *p*-anisidine phosphate, and (*c*) benzidine periodate. G.l.c. analyses were performed on a column of 3% of ECNSS-M and 3% of OV-225 coated on Gas Chrom Q (100–200 mesh), at 170°, with a H_2 flame-ionization detector. H.p.l.c. of neutral sugars and alditols used a Waters HPLC (model 244) apparatus equipped with a refractive index detector and a μBondapak/carbohydrate column, using 3:17 H_2O–MeCN as the mobile phase. Ultracentrifugation was performed with a Beckman analytical ultracentrifuge, using a standard, double-sector, centrifuge cell with a 12-mm filled Epon centerpiece. Demineralizations were effected with freshly regenerated cation (Zeokarb 225H) and anion (Deacidite FS-IP) exchange resins. The emulsin enzyme used was extracted from almonds.

Isolation.—Brown, matured, *P. aculeata* seeds were cleaved and dried, and then defatted with light petroleum (60–80°). The defatted material (100 g) was soaked overnight in water (1.5 L), and then stirred for 2 h at 40–45°. The aqueous extract was filtered, and the filtrate acidified to pH 5 with AcOH and centrifuged. The clear solution was poured into EtOH (4 vol.). After decantation of the EtOH, the precipitate was successively triturated with Me_2CO and absolute EtOH until the precipitate became granular. The crude polysaccharide (11 g, sulfated ash 2.2%) was purified *via* copper complexation[13] (10.6 g, sulfated ash 0.88%). The polysacchar-

TABLE II

DATA FOR OLIGOSACCHARIDES[a] **1–5**

Oligosaccharide	*R*[b]	$[\alpha]_D^{30c}$ *(degrees)*	*D.p.*	*Sugar component (with ratio)*	IO_4^- *consumed*[d]	HCO_2H *liberated*[d]	*Glycol composition*	*Methylated sugars and their ratio*
1 β-Man*p*-(1→4)-Man	1.27	8.3	1.90	D-mannose	2.96	0.95	1:1, erythritol and glycerol	—
2 β-Man*p*-(1→6)-Man	1.80	−12.4	1.80	D-mannose	4.02	1.98	glycerol	—
3 α-Gal*p*-(1→6)-Man	1.10	+124.2	1.83	1:1, D-galactose, D-mannose	3.89	1.92	glycerol	—
4 β-Man*p*-(1→4)-β-Man*p*-(1→4)-Man	0.62	−14.0	2.95	D-mannose	4.06	1.05	1:2, erythritol and glycerol	2,3,4,6,-Me_4-D-Man, 2,3,6-Me_3-D-Man (1:2)
5 α-Gal*p*-(1→6)-β-Man*p*-(1→4)-Man	0.58	94.4	3.04	1:2, D-galactose, D-mannose	5.88	1.92	2:1,erythritol and glycerol	2,3,4,6-Me_4-D-Gal, 2,3,4-Me_3-D-Man, 2,3,6-Me_3-D-Man (1:1:1)

[a] Characteristic values of oligosaccharides **1–5** are in good agreement with literature data[23–26]. [b] Migration rate relative to cellobiose in solvent *D*. [c] For solution in water. [d] Mol/mol of methyl glycoside of oligosaccharide.

TABLE III

DATA FOR METHYLATED SUGARS OBTAINED FROM METHYLATED *P. aculeata* GALACTOMANNAN

Fraction/ Peak	*O-Methyl sugar*[a]	R_{TMG}[b] *Solvent*		$[\alpha]_D^{28}$	*OMe(%)*	R_f[c]		*Molar ratio*[d]	
		A	*C*	*(degrees)*		*3% ECNSS-M*	*3% OV-225*	*X*	*Y*
1	2,3,4,6-Me_4-D-Man	0.98	1	−2.4	52.6	1.00	0.98	1	1
2	2,3,4,6-Me_4-D-Gal	0.89	0.87	116.8	52.2	1.25	1.18	3.04	3.00
3	2,4,6-Me_3-D-Man	0.84	0.71		41.2	2.09	1.89	1.01	1.05
4	2,3,6-Me_3-D-Man	0.81	0.64	−6.5	41.4	2.20	2.02	5.15	3.08
5	2,4,6-Me_3-D-Gal	0.67	0.48		41.9	2.28	2.02		2.04
6	2,3-Me_2-D-Man	0.54	0.28	−16.8	29.6	4.75	3.85	3.96	3.92

[a]As alditol acetates. [b]Migration rate relative to 2,3,4,6-Me_4-Glc in solvents (A) and (C); the values are in good agreement with literature data [27,28]. [c]Retention times relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol; the values are reported in the literature[29,30]. [d]Relative to 2,3,4,6-tetra-*O*-methyl-D-mannose. X: by g.l.c. on column of 3% OV-225; Y: by g.l.c. on column of 3% ECNSS-M.

ide, finally obtained conventionally, had $[\alpha]_D^{20}$ $-5.5 \rightarrow +3.5°$ (1% in tris(hydroxymethyl)aminomethane buffer, pH 7.4) and sulfated ash 0.24%.

Homogeneity.— Upon paper electrophoresis[5], the borate complex of the polysaccharide moved as a single component, and its ionic mobility (μ) in 0.05M sodium tetraborate buffer (pH 9.2) under a field strength of 50 V/cm for 5 h was determined to be 0.012×10^{-5} cm^2. V^{-1}.s^{-1}. During free-boundary electrophoresis[6], only that single peak was recorded photographically at 20, 25, 30, and 35 min. The homogeneity of the polysaccharide was finally established from ultracentrifugal analysis[7,8] at various polysaccharide concentrations (0.3 to 0.8 g/dL) in 0.1M sodium chloride at a speed of 52,000 r.p.m. Only a single boundary in the Schlieren velocity pattern was observed at different time-intervals.

Molecular weight.—The M_w value of the polysaccharide was determined by sedimentation equilibrium over a concentration range from 0.4 to 1.0 g/dL in 0.1M NaCl at 6960 r.p.m. and a temperature of 25°. The refractive index distributions were measured at equilibrium with Rayleigh interference optics and apparent M_w values were calculated. The M_w value was determined by extrapolation of the curve of concentration versus $1/M_w$(app) to zero concentration.

Hydrolysis.—Purified polysaccharide (0.1 g) was hydrolyzed with 2M CF_3CO_2H (20 mL) in a sealed tube for 3 h at 100°. Identification and quantitation of monosaccharides were performed by h.p.l.c., and by g.l.c. of the alditol acetates (see Table I).

Partial hydrolysis with acid.—The polysaccharide (2 g) was hydrolyzed with 0.25M CF_3CO_2H (50 mL) for 7 h at 100°. After removal of the acid with MeOH, the hydrolyzate was resolved by preparative p.c. in solvent *D*. The degree of polymerization of each homogeneous oligosaccharide was determined by the anthrone-H_2SO_4 method[14]. Experimental data for oligosaccharides **1**–**5** are summarized in Table II.

Periodate oxidation.—The course of periodate oxidation[15,16] of the polysaccharide was monitored iodometrically[17,18] up to 120 h. After reduction with $NaBH_4$ of the oxopolysaccharide thus obtained, and hydrolysis with H_2SO_4 (0.5M, 20mL) for 10 h at 100°, the hydrolyzate showed four spots on p.c. with solvent *A* and detection reagent *a*. Identification and quantitation[19,20] revealed galactose, mannose, erythritol, and glycerol in the molar ratios of 2:1:7:4. The molar proportions were confirmed by h.p.l.c. analysis.

Methylation.—The polysaccharide (2.5 g) was completely methylated by the Haworth[10] and Hakomori[11] methods. The permethylated polysaccharide (1.8 g; OMe 45.28%) was hydrolyzed by the method[21] of Croon *et al.* The methylated sugar analyses were preformed by p.c. and g.l.c. (see Table III).

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