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STRUCTURE OF A GALACTOMANNAN FROM *Cassia alata* SEED*

DHIRENDRA S. GUPTA**, BARBARA JANN,
Max-Planck-Institut für Immunbiologie, 7800 Freiburg (F.R.G.)

KRISHNA S. BAJPAI,
Chemistry Department, P.P.N. College, Kanpur, U.P. (India)

AND SATISH C. SHARMA
Chemistry Department, D.S. College, Aligarh, U.P. (India)

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ABSTRACT

A water-soluble galactomannan, isolated from the seeds of *Cassia alata* Linn, has been investigated by using methylation analysis, periodate and CrO_3 oxidation, n.m.r. spectroscopy, and reaction with *Bandeiraea simplicifolia* lectin and an α-D-galactosidase. The polysaccharide is composed of heptasaccharide units joined by β-(1→4) linkages. The polysaccharide has a molecular weight of 26,400, corresponding to ~23 units.

INTRODUCTION

Plant galactomannans are of industrial importance[1,2], notably in food, pharmaceuticals, cosmetics, paper products, paints and plastics, well drilling and mining, and explosives. Some variously interact with milk proteins, plant lectins[3], and protein antibodies[4], possess non-cytotoxic antitumor activity[5], act as inhibitors of viruses[2], and induce interferon in cell cultures[6]. We report now on the structure of a polysaccharide isolated from *Cassia alata* seeds.

RESULTS AND DISCUSSION

The crude product, extracted with water from *Cassia alata* seeds, was fractionated by precipitation with ethanol and gave a polysaccharide fraction (70% yield) with 50% aqueous ethanol. The minor fractions obtained at lower and higher concentrations of ethanol were not studied further. The polysaccharide had $[\alpha]_D^{25}$

*Dedicated to Professor Dr. Klaus Jann.

**Author for correspondence. Present address: Chemistry Department, V.S.S.D. Post-Graduate College (Kanpur University), Kanpur 208 002, U.P., India.

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TABLE I

RETENTION TIMES AND MASS-SPECTRAL DATA OF THE PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM *C. alata* POLYSACCHARIDE

Peak	T_R[a]	*Ratio*[b]	*Characteristic mass ions* (m/z)	*Structure assigned*
A	1.25	0.89	45, 117, 161, 205	1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol
B	2.41	1.50	45, 117, 233	1,4,5-Tri-*O*-acetyl-2,3,6-tri-*O*-methylmannitol
C	4.81	1.00	117, 261	1,4,5,6-Tetra-*O*-acetyl-2,3-di-*O*-methylmannitol

[a]Retention time in g.l.c. relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on SE-30. [b]Calculated taking the amount of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylmannitol as unity.

+24° (water), gave a single peak on elution from Sepharose 6B, and gave a symmetrical peak on ultracentrifugation. The molecular weight, determined according to Yphantis[7], was 26,400. Acid hydrolysis of the polysaccharide yielded galactose (26.6%) and mannose (71.8%) in the molar ratio 1:2.69, and their optical rotations indicated that they were D sugars.

The results obtained on methylation analysis of the polysaccharide are given in Table I and indicate that the backbone comprises (1→4)-linked mannosyl residues to which single galactosyl groups are linked at positions 6 (formation of 2,3-di-*O*-methylmannose).

Acid hydrolysis of the product obtained on borohydride reduction of the periodate-oxidised polysaccharide gave (p.c.) glycerol, erythritol, and mannose. Conversion of the products of acid hydrolysis into alditol acetates and g.l.c. analysis showed that 46% of the original mannose had survived. The results of methylation analysis (60% of 4-linked mannose, 40% of 4,6-linked mannose) do not agree with those of the periodate oxidation. However, further destruction of mannose was observed after prolonged periodate oxidation (20% residual mannose after 140 h and 15% after 236 h). Thus, periodate oxidation of the backbone is hindered by the side chains. Methylation analysis of the periodate-oxidised (236 h) and borohydride-reduced polysaccharide gave 2,3,6-tri- and 2,3-di-*O*-methylmannoses. Thus, not only the branch-point mannose but also some unsubstituted mannose escaped oxidation. The resistance of mannosyl residues to periodate oxidation has been observed by others[8–12]. The release of glycerol and erythritol on acid hydrolysis of the polyalcohol corroborates the results of methylation studies.

The ^{1}H-n.m.r. spectrum of the polysaccharide contained signals for H-1 of Gal (δ 4.98, d, $J_{1,2}$ ~3.6 Hz) and Man (δ 4.69) compatible with the expected $^4C_1$ conformation of the α-D-galactopyranose and β-D-mannopyranose rings (Fig. 1A).

TABLE II

^{13}C-N.M.R. DATA FOR THE GALACTOMANNAN FROM *C. alata* SEED

Unit	*Chemical shift*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-D-Galactopyranosyl	100.1	70.6	69.70	70.7	72.6	62.5
4-Linked β-D-mannopyranosyl	101.5	71.2	72.80	77.8	76.3	61.9
4,6-Linked β-D-mannopyranosyl	101.3	71.2	72.80	78.1	74.7	67.9

[a]In p.p.m., relative to the signal for Me_4Si.

The ^{13}C-n.m.r. data for the polysaccharide (Table II) indicate substitution of mannose at C-4 and C-6 [α shifts: C-4, Δ 10.7 (4-linked) and 11.0 (4,6-linked); C-6, Δ 6.5 (4,6-linked)][13]. The chemical shift assignments accord with those reported[14,15].

The anomeric configuration of the residues was determined by a gated decoupling experiment [α-Gal $J_{C\text{-}1,H\text{-}1}$ 169.8 Hz; β-Man $J_{C\text{-}1,H\text{-}1}$ 162.5 Hz] and by oxidation

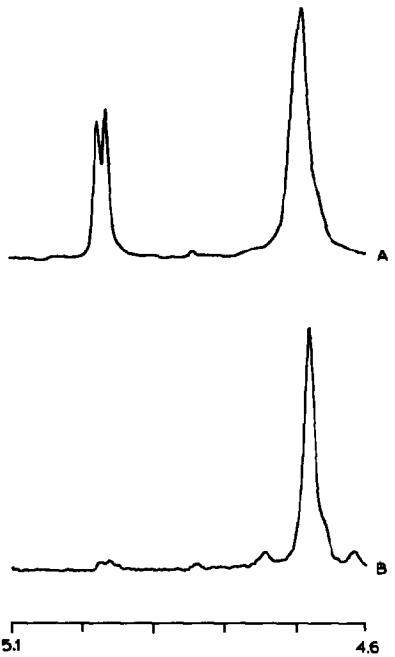

Fig. 1. ^{1}H-N.m.r. spectra (300 MHz) of solutions of *C. alata* galactomannan (10 mg/mL) in D_2O at 70°: A, native polysaccharide; B, after treatment with α-D-galactosidase.

of the acetylated polysaccharide with chromium trioxide (β residues are oxidised at a higher rate than the α residues[16]). Whereas >70% of the galactose residues survived, >97% of mannose residues were destroyed, suggesting that the D-mannose was β and the D-galactose was α. That the D-galactose residues were α was confirmed (*a*) by the strong precipitin reaction of the polysaccharide and *Bandeiraea simplicifolia* lectin which is specific for α-D-galactosyl residues[17] and (*b*) by treatment of the polysaccharide with α-D-galactosidase which hydrolysed almost all of the galactosyl residues and gave a mannan-type polymer (Fig. 1B).

Thus, it is concluded that the galactomannan obtained from *C. alata* seeds consists of a backbone of (1→4)-linked β-D-mannopyranosyl residues with α-D-galactopyranosyl groups attached to positions 6, a feature common to most galactomannans of leguminous seed[18]. The polysaccharide contains one D-galactose residue per ~2.5 D-mannose residues. The distribution of the D-galactose residues remains to be determined.

EXPERIMENTAL

Analytical methods. — Evaporations were conducted under diminished pressure at <45° (bath). Descending p.c. and t.l.c. were done on Whatman No. 1 and 3MM papers and silica gel (Merck), respectively, using 1-butanol–pyridine–water (6:4:3). Sugars were detected by aniline hydrogenphthalate[19] and alkaline silver nitrate[20]. Constituent sugars were determined as the alditol acetates[21] by g.l.c. with ribose as the internal standard, using a Varian Aerograph series 1400 chromatograph fitted with an automatic recorder, a temperature programmer, a 3380 A Hewlett–Packard integrator, and a 3% ECNSS-M column. N.m.r. spectra were recorded on a Bruker WH-300 instrument. For the ^{1}H-n.m.r. spectroscopy at 70°, the sample (10 mg) was repeatedly dissolved in D_2O (5 × 5 mL) and the solution was lyophilised. The final freeze-dried sample was dissolved in 1 mL of 99.99% D_2O. For ^{13}C-n.m.r. spectroscopy at 50°, the sample (65 mg/mL) was dissolved in D_2O (external sodium 3-trimethylsilylpropionate-2,2,3,3-d_4). The ^{13}C chemical shifts were corrected (−1.31 p.p.m.) by reference to 1,4-dioxane (δ 67.4 relative to Me_4Si). The ^{1}H chemical shifts were corrected likewise (−0.07 p.p.m.); dioxane signal at δ 3.7). Analytical centrifugation was carried out at 20°, using a Beckman Model E Spinco analytical ultracentrifuge. A solution (6 mg/mL) of the polysaccharide in 0.2M ammonium acetate was placed in a single sector cell (12 mm, 4°). The sedimentation coefficient was calculated by the method of Schachmann[22] and expressed in Svedberg units (S = 10^{-13} s), and the molecular weight by the Yphantis method[7]. Optical rotations were measured with a Perkin–Elmer 141 polarimeter.

Isolation and purification of the polysaccharide. — *Cassia alata* seeds (500 g) were cleaved at low speed in a hand mill, and the cotyledons were separated from the seed coats (250 g) through winnowing. The seed coats (100 g) were soaked in water overnight at 4°, and the polysaccharide was extracted by heating the contents

to 40–50° for 1 h, crushing the swollen material, and filtration. The resulting viscous solution was centrifuged (15,000*g*), the crude polysaccharide was precipitated from the supernatant solution by adding 1 vol. of ethanol and collected by centrifugation, and a solution in water was freeze-dried.

The crude polysaccharide was purified by fractional precipitation from a 0.6% aqueous solution with ethanol.

Investigation of the polysaccharide. — (*a*) *Sugar composition.* The polysaccharide was hydrolysed with M hydrochloric acid in a sealed tube for 2.5 h at 100°. The hydrolysate was processed in the usual manner, and the components were identified and their ratios determined by g.l.c. of their alditol acetates.

(*b*) *Gel filtration.* A solution of the polysaccharide (2 mg) in 0.2M sodium chloride (1 mL) was applied to a column (1.5 × 45 cm) of Sepharose 6B and eluted with 0.2M sodium chloride at 10 mL/h. Fractions (1 mL) were collected and analysed by the phenol–sulphuric acid method[23].

(*c*) *Methylation analysis.* The polysaccharide (6 mg) was methylated by a modified Hakomori method[24] and then hydrolysed with $HCOOH/H_2SO_4$, and the products were converted into the partially methylated alditol acetates[25].

(*d*) *Periodate oxidation.* A solution of the polysaccharide (25 mg) and Sörensen phosphate buffer (pH 7.0) was oxidised with 0.06M sodium metaperiodate at 4° in the dark for 100, 140, and 236 h. Excess of periodate was reduced by ethylene glycol (2 mL), the mixture was dialysed for 46 h against distilled water and then concentrated under reduced pressure (to ~30 mL), and the products were reduced overnight with sodium borohydride (65 mg) at room temperature. The excess of borohydride was destroyed by Dowex 50-X8 (H^+) resin, the solution was filtered, and the polyalcohol (22 mg) was recovered by dialysis and freeze-drying. Samples of this material were subjected to sugar analysis and methylation analysis.

(*e*) *Oxidation with chromium trioxide.* The acetylated[26] polysaccharide (11 mg) and *myo*-inositol hexa-acetate (2.4 mg) were dissolved in chloroform (1 mL), and a portion (0.3 mL) of this solution was taken as a control. The remaining solution was concentrated to dryness, glacial acetic acid (4 mL) and chromium trioxide (60 mg) were added to the residue, and the mixture was kept at 50° with sonication for 90 min. The product was recovered by partition between chloroform and water. Sugar analysis was carried out before and after oxidation. The ratios of *myo*-inositol, mannose, and galactose in the control and oxidised material were 1.0:0.49:0.2 and 1.0:0.013:0.14, respectively.

(*f*) *Precipitin reaction.* Aliquots (5, 10, and 20 μL) of a 3% solution of the polysaccharide in phosphate-buffered saline (PBS, pH 7.33) were applied to three wells in an agarose gel plate (1.2% of agarose in PBS). In the central well, 10 μL of 0.3% solution of *Bandeiraea simplicifolia* lectin[17] (Vector Laboratories, Inc., gift from Dr. Hubert Mayer of this Institute) in PBS was applied. The plate was kept at 4° in the cold room overnight and the appearance of precipitin bands was observed.

(*g*) *Treatment with α-D-galactosidase.* To a solution of the polysaccharide (12

mg) in Sörensen phosphate buffer (pH 6.5; 2 mL) was added 40 μL of α-D-galactosidase suspension (5 mg/mL; from coffee beans, Boehringer Mannheim). The mixture was stirred and dialysed at 37° overnight against the same buffer. The dialysed solution was heated to ~100° for 5 min to destroy the enzyme and then centrifuged, and the supernatant solution was dialysed against distilled water and freeze-dried. The ^{1}H-n.m.r. spectrum of freeze-dried material (7 mg) is shown in Fig. 1B. The dialysate was deionised using ion-exchange resins and concentrated to a syrup. T.l.c. and p.c. of the syrup revealed galactose only.

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