Carbohydrate Research, 182 (1988) 119–125
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

HETEROGENEITY AND STRUCTURAL INVESTIGATION OF GALACTOMANNANS ISOLATED FROM THE SEEDS OF *Cassia sericea*

GUDIPATI MURALIKRISHNA, RUDRAPATNAM N. THARANATHAN*,
Biochemistry Section, Department of Food Chemistry, Central Food Technological Research Institute, Mysore 570 013 (India)

AND U. RAMADAS BHAT
Max-Planck-Institut für Immunobiologie, Stübeweg 51, 78 Freiburg-Zähringen (F.R.G.)

(Received February 4th, 1988; accepted for publication, March 16th, 1988)

ABSTRACT

Two homogeneous galactomannans were isolated from the seeds of *Cassia sericea* and investigated by methylation analysis, periodate and CrO_3 oxidation, ^1H- and ^{13}C-n.m.r. spectroscopy, and reaction with α-D-galactosidase. The polysaccharides had backbones of (1→3)- and occasional (1→4)-linked β-D-mannopyranosyl residues and side chains, at positions 6, of single α-D-galactopyranosyl groups. One of the polysaccharides also had a few non-reducing terminal mannosyl groups.

INTRODUCTION

The endosperm of many plant seeds (mainly belonging to the family *Leguminoseae*) contains galactomannans which have attracted much attention, and the ratio of mannose and galactose, the nature and distribution of the galactose side-chains along the mannose backbone, and the d.p. affect the solubility, gelling, and functional characteristics[1–3]. Seeds of several *Cassia* species have been investigated[4–12] and we now report on the polysaccharides isolated from the seeds of *Cassia sericea*. This plant is reported to arrest the growth and spread of the noxious weed *Parthenium*, the pollen of which causes allergy in humans[13].

RESULTS AND DISCUSSION

Aqueous extraction of the 60-mesh powder of the seeds furnished cold-water-soluble (CWS) and hot-water-soluble (HWS) polysaccharides in yields of 65 and 24%, respectively. The crude polysaccharides were composed mainly of mannose and galactose (Table I). Only 83% of CWS redissolved in water and the insoluble

*Author for correspondence.

0008-6215/88/$ 03.50

TABLE I

SUGAR COMPOSITION (%) OF *C. sericea* POLYSACCHARIDE AND ITS FRACTIONS

	Native	*CWS*	*HWS*	*Water-insoluble residue*	*Fractions*[a]			
					1	*2*	*3*	*4*
Yield (%)	(100)	65	24	17.5	14	28	17	12
Sugars identified[b]								
Rha	1.7	0.1	0.4	17.2	9.9	5.0	1.0	8.4
Ara	10.5	12.1	19.2	16.6	39.2	5.2	4.6	16.7
Xyl	3.6	3.2	5.8		5.0		0.6	15.6
Man	54.6	54.2	51.7	12.1	28.6	61.0	66.0	43.3
Gal	23.1	30.5	23.3	54.2	17.2	28.9	27.8	15.9

[a]Derived from the water-soluble portion of CWS (see Experimental). [b]Quantified by g.l.c. as alditol hexa-acetates, using *myo*-inositol as the internal standard.

residue was an arabinogalactan (Table I), probably originating from the hemicellulosic fraction(s) of the seed coat.

Fractionation of the water-soluble portion of CWS with aqueous 7% cupric acetate gave four sub-fractions of differing sugar composition (Table I) in yields of 12–28%. Fractions 2 (28%) and 3 (17%) each gave a single symmetrical peak on elution from Sephacryl S-300 and also on ultracentrifugation and electrophoresis, had $[\alpha]_D$ values of +30° and +28° (water), respectively, and were composed mainly of D-galactose and D-mannose in the molar ratios 1:2.11 and 1:2.36, respectively. These ratios, deduced[14] from the relative peak areas of the signals for H-1 (1:1.97 and 1:2.25) and C-1 (1:1.98 and 1:2.67), were reasonably comparable with those noted above, calculated from the g.l.c. data. Molecular sieving on Sephacryl S-300 indicated fractions 2 and 4 to have molecular weights of 1.78×10^5 and 1.1×10^5, respectively.

The results of methylation analysis (Table II) indicated fractions 2 and 3 each to contain a backbone mainly composed of (1→3)- (~80%) and (1→4)-linked (~20%) D-mannosyl residues. The side chains attached to positions 6 of mannosyl residues contained single D-galactosyl groups. Fraction 3, in addition, contained small amounts of non-reducing terminal mannopyranosyl groups, a feature not reported hitherto for seed galactomannans[2].

Supporting the results of methylation analysis, mild hydrolysis with acid of the product obtained on borohydride reduction of the periodate-oxidised polysaccharides gave glycerol, erythritol, and mannose. G.l.c. quantification of the latter showed that ~80% of the original mannose had survived oxidation.

The partial ^{1}H-n.m.r. spectrum of fraction 2 is shown in Fig. 1. Although broad, the resonances of the anomeric protons are well separated. The doublet at 4.97 p.p.m. from H-1 of Gal, however, has $J_{1,2}$ ~2.8 Hz, which is slightly less than the reported[14] value of 3.0 Hz, and the signal for H-1 of Man at ~4.69 p.p.m. ($J_{1,2}$

TABLE II

G.L.C.-M.S. DATA OF THE PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM FRACTIONS 2 AND 3

Peak	T_R^g	*Molar ratio*[b]	*Diagnostic ions* (m/z)	*Structure deduced*
Fraction 2				
1	1.18	1.00	45, 117, 161, 205	2,3,4,6-Tetra-*O*-methylgalactitol
2	1.92	1.50	45, 117, 161, 173, 233	2,4,6-Tri-*O*-methylmannitol
3	3.71	0.69	117, 161, 201, 261	2,3-Di-*O*-methylmannitol
Fraction 3				
1	1.00	0.16	45, 117, 161, 205	2,3,4,6-Tetra-*O*-methylmannitol
2	1.17	1.00	45, 117, 161, 205	2,3,4,6-Tetra-*O*-methylgalactitol
3	1.92	1.68	45, 117, 161, 173, 233	2,4,6-Tri-*O*-methylmannitol
4	3.70	0.92	117, 161, 201, 261	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 OV-225. [b]Relative to 2,3,4,6-tetra-*O*-methylgalactitol.

~0.9 Hz) is close to the reported[15,16] values, and therefore compatible with the expected $^4C_1$ conformation of the α-D-galactopyranose and β-D-mannopyranose rings. The ^{1}H-n.m.r. spectrum of fraction 3, although comparable to that of fraction 2, was not so sharp for reasons unknown at present.

The broad-band-decoupled ^{13}C-n.m.r. spectrum (Fig. 2) confirms the substitution pattern of mannose revealed by the methylation studies. All of the resonances are resolved[17] and their chemical shifts are recorded in Table III. The anomeric configuration of the residues was determined by a gated decoupling experiment which gave $J_{C\text{-}1,H\text{-}1}$ values for α-Gal and β-Man of 174.5 and 158.5 Hz, respectively.

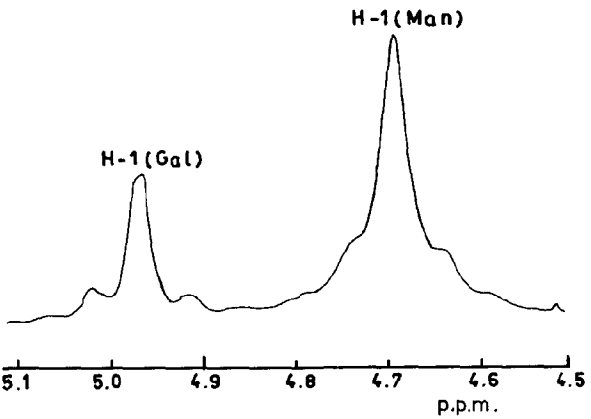

Fig. 1. Partial ^{1}H-n.m.r. spectrum (300 MHz) of a solution (15 mg/mL in D_2O) of *Cassia sericea* galactomannan fraction 2 at 70°.

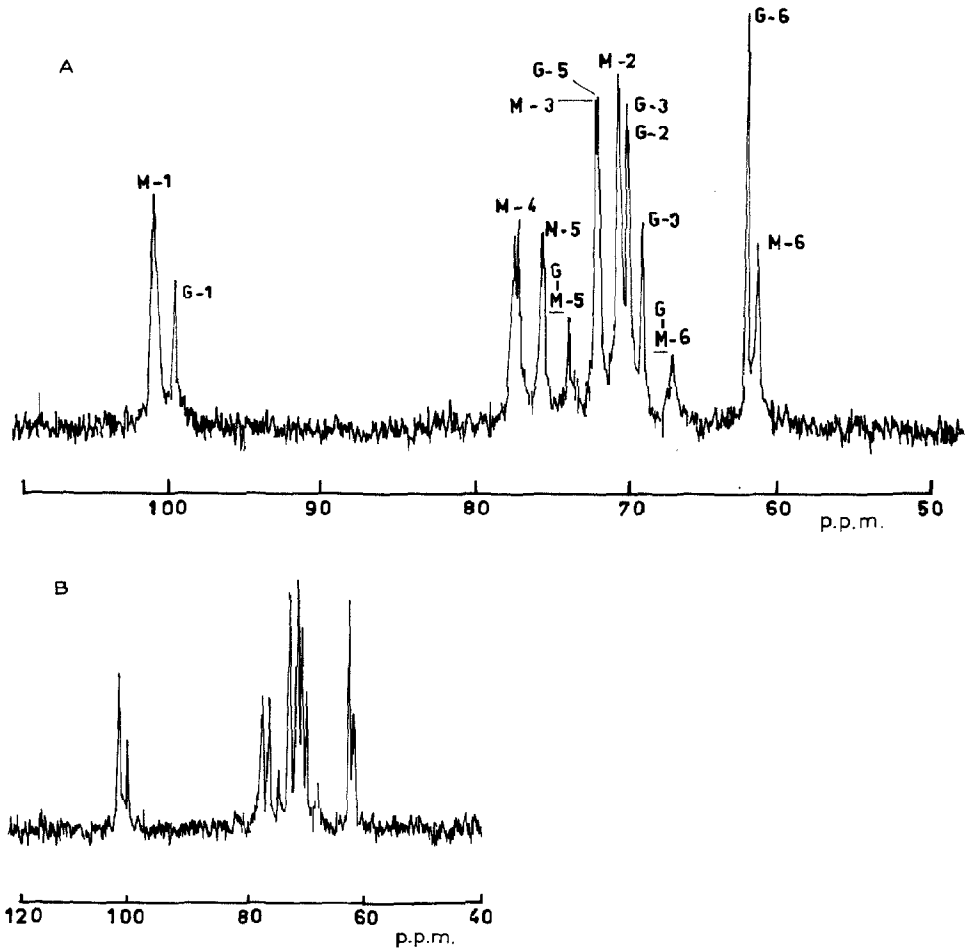

Fig. 2. ^{13}C-N.m.r. spectra (300 MHz) of solutions (50 mg/mL in D_2O at 50°) of *Cassia sericea* galactomannans: A, fraction 2; B, fraction 3.

A similar inference was made from the effect of chromium trioxide on the acetylated polysaccharides[18] and by treatment of the polysaccharides with α-D-galactosidase[2]. In the former, there was almost complete destruction of the mannose residues but >90% of the galactose residues survived, suggesting[18] that the D-mannose was β and the D-galactose was α. In the latter, enzymic hydrolysis of almost all of the D-galactosyl residues confirmed the presence in fractions 2 and 3 of α-D-galactosyl residues. The enzyme-treated polysaccharide was composed exclusively of mannose.

Thus, the galactomannans isolated from the seeds of *C. sericea* have an "anomalous" backbone of mainly (1→3)-linked together with a small proportion of (1→4)-linked β-D-mannopyranosyl residues with single α-D-galactopyranosyl units

TABLE III

^{13}C-N.M.R. DATA FOR THE GALACTOMANNAN (FRACTION 2) FROM *C. sericea* SEED

Type of unit	*Chemical shift*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-D-Gal*p*	99.5	70.0	69.2	70.6	72.0	62.0
3-linked β-D-Man*p*	100.9	70.7	72.2	77.5	75.8	61.3
3,6-linked β-D-Man*p*	100.8	71.8	72.5	77.7	75.7	69.4

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

attached to positions 6. The distribution of these side chains remains to be determined. The galactomannans so far reported possess a (1→4)-linked β-D-mannan backbone[2], except for that of *C. absus* seed[5,6], which contains ~20% of (1→3)-linked mannose residues 50% of which are 2-substituted by α-D-galactosyl groups, and the remainder of the α-D-galactosyl units are attached to positions 6 of the backbone. Such unusual structures are similar to those characterised in micro-organisms[19]. The galactomannans from *Crotalaria mucronata*[20] and *Caesalpinia pulcherima*[21] have both (1→3)- and (1→4)-linked mannose in the backbone.

Polydispersity in galactomannans has been seldom reported, except for that from *C. absus*[5,6] which was shown to be a mixture of more than two polysaccharides. In view of their almost similar ratios of D-Man and D-Gal and only small differences in molecular weight, it is likely that, in the galactomannans from *C. sericea*, there may be variations in the distribution of galactose along the main chain.

EXPERIMENTAL

Isolation and purification of the polysaccharide. — *Cassia sericea* seeds (210 g, obtained from the University of Agricultural Sciences, Hebbal, Bangalore) were powdered (60 mesh) in a hand mill and then defatted (and depigmented) by successive treatments with boiling chloroform–hexane (1:1) followed thrice by extraction with aqueous 70% ethanol. The product was extracted with water at room temperature and 85°. Brief centrifugation of the viscous extracts followed by addition of ethanol (3 vol.) to the supernatant solutions precipitated the crude polysaccharides CWS and HWS, respectively. CWS (22 g) was insoluble in water to the extent of 17.5%. To the soluble portion was added aqueous 7% cupric acetate (15 mL), and the precipitate formed was collected by centrifugation. Further additions of cupric acetate to the clear supernatant solution yielded three more fractions. Each time, the cupric complex was dissociated by macerating with acidic ethanol and the polysaccharide was recovered.

Homogeneity criteria. — *(a) Gel filtration.* A solution of the polysaccharide (5

mg) in 0.2M sodium chloride (1 mL) was applied to a column (1.5 × 87 cm) of Sephacryl S-300 and eluted with 0.2M sodium chloride at 7.5 mL/h. Fractions (1 mL) were analysed by the phenol–sulphuric acid method.

(b) Sedimentation. The sedimentation behaviour of a solution of the polysaccharide (2 mg) in 0.1M sodium chloride (1.5 mL) at 56,000 r.p.m. was determined in a Beckman analytical ultracentrifuge.

(c) Electrophoresis. Microzone electrophoresis of the dyed (Procion Brilliant Red 2BS) polysaccharides[22] on cellulose acetate membranes was carried out in a Beckman microzone cell in acetate buffer (0.05M, pH 4.8) at an applied voltage of ~180V.

Methylation analysis. — The polysaccharides were methylated by the Hakomori method[23], using potassium hydride–MeI, and the products were purified by passing through SEP-PAK C_{18} cartridges. After hydrolysis with formic acid–sulphuric acid, the products were converted into partially methylated alditol acetates and analysed[24] by g.l.c.–m.s.

Periodate oxidation. — A solution of the polysaccharide (10 mg in 10 mL of water) was oxidised with 0.05M sodium metaperiodate for 48 h in the dark at 4°. The excess of periodate was reduced with 0.1M ethylene glycol (2 mL) and the products were reduced with sodium borohydride (25 mg). The solution was then dialysed and freeze-dried. The resulting polyalcohol was hydrolysed with 0.5M sulphuric acid for 48 h at room temperature followed by p.c. and by g.l.c. of the alditol acetates.

Chromium trioxide oxidation. — The polysaccharide (20 mg) was acetylated with acetic anhydride in formamide. To a portion of the product in glacial acetic acid (2 mL) was added chromium trioxide (50 mg), and the oxidation at room temperature was continued for 4 h. The products were recovered by partition between chloroform–water and sugars analysed by p.c. and g.l.c. both before and after oxidation[18].

Reaction with α-D-galactosidase. — To a solution of the polysaccharide (10 mg) in 0.05M acetate buffer (pH 5.2, 2 mL) was added 1 mL of α-D-galactosidase (Sigma) in acetate buffer (0.5 U), and the mixture was incubated for 48 h at 37°. Ethanol (3 vol.) was added and the precipitated polysaccharide was separated by a brief centrifugation. The galactose in the supernatant solution was analysed by p.c. and by g.l.c. of the derived galactitol hexa-acetate. Also, the enzyme-treated polysaccharide was hydrolysed with acid and the products were analysed by g.l.c.

General methods. — Most of the general and analytical methods have been described[25,26]. N.m.r. spectra were recorded on solutions in D_2O with a Bruker WM-300 spectrometer in the pulsed F.t. mode. For ^{1}H-n.m.r. spectra at 70°, the sample (15 mg) was repeatedly dissolved in D_2O (3 × 2 mL) and the solution lyophilised. The resulting sample was dissolved in 1 mL of 99.99% D_2O. ^{13}C-N.m.r. spectra at 50° were accumulated with and without proton decoupling. The external standard was sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)propionate. The ^{13}C chemical shifts were corrected (−1.31 p.p.m., relative to external Me_4Si, 67.4). The ^{1}H

chemical shifts were similarly corrected (−0.07 p.p.m.) relative to external 1,4-dioxane, 3.7. Optical rotations were measured on solutions in water (0.5%) with a Perkin–Elmer model 243 polarimeter.

ACKNOWLEDGMENTS

We thank Professor M. Mahadevappa (The University of Agricultural Sciences, Hebbal, Bangalore) for the generous supply of *C. sericea* seeds, D. Borowiak for the g.l.c.–m.s., and H. Kochanowski for n.m.r. spectroscopy.

REFERENCES

1 R. N. THARANATHAN, G. MURALIKRISHNA, P. V. SALIMATH, AND M. R. RAGHAVENDRA RAO, *Proc. Indian. Acad. Sci. (Plant Sci.)*, 97 (1987) 81–155.
2 P. M. DEY, *Adv. Carbohydr. Chem. Biochem.*, 35 (1978) 341–376.
3 R. L. WHISTLER (Ed.), *Industrial Gums*, Academic Press, New York, 1959.
4 P. S. KELKAR AND S. MUKHERJEE, *Indian J. Chem.*, 9 (1971) 1085–1087.
5 V. P. KAPOOR AND S. MUKHERJEE, *Can. J. Chem.*, 47 (1969) 2883–2887.
6 V. P. KAPOOR AND S. MUKHERJEE, *Indian J. Chem.*, 10 (1972) 155–158.
7 D. S. GUPTA, B. JANN, K. S. BAJPAI, AND S. C. SHARMA, *Carbohydr. Res.*, 162 (1987) 271–276.
8 A. K. SEN, K. K. SARKAR, N. BANERJEE, AND M. I. H. FAROOQUI, *Indian J. Chem.*, 26B (1987) 21–25.
9 S. A. I. RIZVI, P. C. GUPTA, AND R. K. KAUL, *Planta Med.*, 20 (1971) 24–32.
10 K. TEWARI, N. KHARE, V. SINGH, AND P. C. GUPTA, *Carbohydr. Res.*, 135 (1984) 141–146.
11 G. ISLAM, V. P. KAPOOR, AND M. I. H. FAROOQUI, *Sci. Cult.*, 43 (1987) 316–318.
12 R. GUPTA, N. KHARE, V. SINGH, AND P. C. GUPTA, *Carbohydr. Res.*, 159 (1987) 336–340.
13 M. MAHADEVAPPA, personal communication.
14 H. GRASDALEN AND T. J. PAINTER, *Carbohydr. Res.*, 81 (1980) 59–66.
15 T. J. PAINTER, J. J. GONZALEZ, AND P. C. HEMMER, *Carbohydr. Res.*, 69 (1979) 217–226.
16 J. J. GONZALEZ, *Macromolecules*, 11 (1978) 1074–1085.
17 S. M. BOCIEK, M. J. IZZARD, A. MORRISON, AND D. WELTI, *Carbohydr. Res.*, 93 (1981) 279–283.
18 J. HOFFMAN AND B. LINDBERG, *Methods Carbohydr. Chem.*, 8 (1980) 117–122.
19 P. A. J. GORIN AND J. F. T. SPENCER, *Can. J. Chem.*, 46 (1968) 2299–2304.
20 A. M. UNRAU AND Y. M. CHOY, *Can. J. Chem.*, 48 (1970) 1123–1128.
21 A. M. UNRAU AND Y. M. CHOY, *Carbohydr. Res.*, 14 (1970) 151–158.
22 D. M. W. ANDERSON, A. HENDRIE, J. R. A. MILLAR, AND A. C. MUNRAO, *Analyst*, 96 (1971) 870–874.
23 S. I. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
24 P. E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LONNGREN, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–20.
25 P. V. SALIMATH AND R. N. THARANATHAN, *Carbohydr. Res.*, 106 (1982) 251–257.
26 U. RAMADAS BHAT AND R. N. THARANATHAN, *Carbohydr. Res.*, 148 (1986) 143–147.