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0144-8617(95)00045-3

Carbohydrate Polymers 27 (1995) 229–233

0144-8617/95/$09.50+.00

Research Note

X-ray diffraction studies on some seed galactomannans from India

Virendra P. Kapoor,[a] Henri Chanzy[b] & François R. Taravel*[b]

[a]*National Botanical Research Institute, Lucknow, 226001, India*
[b]*Centre de Recherches sur les Macromolécules Végétales, CNRS, BP 53 X, 38041 Grenoble cedex 9, France*

(Received 6 February 1994; revised version received 10 March 1995; accepted 13 March 1995)

The crystalline structures of four Indian seed galactomannans having mannose to galactose ratios ranging from 1·16 to 2·55 have been investigated by wide-angle X-ray diffraction of oriented films. The orthorhombic lattice constants (Å) of the polysaccharides are as follows: galactomannans from *Trifolium alexandrium*, $a = 9{\cdot}02$, $b = 30{\cdot}80$, $c = 10{\cdot}27$; from *Medicago sativa* (lucerne) $a = 9{\cdot}00$, $b = 30{\cdot}66$, $c = 10{\cdot}24$; from *Cassia siamea*, $a = 9{\cdot}00$, $b = 24{\cdot}81$, $c = 10{\cdot}30$; and from *Cassia saltiana*, $a = 8{\cdot}99$, $b = 24{\cdot}75$, $c = 10{\cdot}30$. In agreement with results already published for other seed gums of commercial importance, the values of the b dimension of the unit cell of galactomannans decrease with decreasing galactose content. The values of the a and c dimensions, as found for other galactomannans, are similar to those of mannan I and mannan II, respectively, and can be understood in terms of conformational restrictions on the mannan backbone imposed by the β-(1 → 4) linkage. This similarity suggests that galactomannans of low and high galactose content have a fundamentally related crystal structure.

INTRODUCTION

The seed galactomannans are important products (Dea & Morrison, 1975) used in various industries worldwide. The growing industrial utility of these gums or modified gums (Yalpani & Sandford, 1987) in the fields of oil recovery, paper and textile industries, has resulted in an impetus in India for intensified research on new sources having varying galactose–mannose ratios and different chemical fine structure. All these gums consist of a linear core of β-(1 → 4)-D-mannose units. They differ from one another in their galactose–mannose ratio as well as their fine structure regarding the distribution of the α-(1 → 6)-linked D-galactose units along the mannan backbone. The polysaccharides obtained from *Trifolium alexandrium* and *Medicago sativa* (lucerne) have 81–86%, respectively, of the core mannose substituted by galactose, whereas in *Cassia siamea* (Khan *et al.*, 1988) and *Cassia saltiana* gums, only 39–44%, respectively, of the main chain is substituted.

*Author to whom correspondence should be addressed.

The aim of this note is to provide 'fingerprints' of a series of Indian galactomannans of various origins and to establish a structural basis for a better understanding of their physical properties. The study presents X-ray diffraction data of oriented polysaccharide films to investigate their chain conformation as well as their tridimensional packing. These diffraction data have been compared with reported results of other seed gums of commercial importance.

MATERIALS AND METHODS

The seeds of *Trifolium alexandrium*, *Medicago sativa* (lucerne), *Cassia siamea* and *Cassia saltiana* were collected in India and identified at the Seed Herbarium of the National Botanical Research Institute, Lucknow. The endosperms of the seeds were separated by wet milling process using 20% ethanol. The endosperms were powdered to 60 mesh and extracted with benzene–ethanol (2:1, v/v) in a soxhlet for 16 h. The crude polysaccharides were isolated by extraction with hot water

and fractional precipitation with ethanol according to a previously described method (Kapoor *et al.*, 1989).

The polysaccharides were further purified as follows: a viscous solution (2% w/v) was prepared under continuous stirring for 8–12 h at 45°C and precipitated with saturated barium hydroxide solution. Each resulting barium complex was centrifuged and decomplexed with 2 N acetic acid under continuous stirring for 12 h. The corresponding polysaccharide was recovered from the centrifuged solution by precipitation with ethanol and washed successively with 60, 70, 80, 90 and 95% ethanol. The product was redissolved in distilled water (1 g/l), and dialysed with running distilled water for 48 h followed by concentration and freeze-drying.

Polysaccharide samples were hydrolysed with 1 N trifluoroacetic acid (4 h, 100°C) followed by reduction with sodium borohydride and acetylation with pyridine–acetic anhydride (1:1 v/v, 1 h, 100°C). The resulting alditol acetates were analysed by GLC using a Supelco SP 2380 column (30 m × 0·53 mm) with a temperature programme from 195°C (4 min) to 225°C at a rate of 2·5°C/min. Gas chromatography–mass spectrometry (GC–MS) spectra were recorded with a Nermag R 1010C spectrometer equipped with a Delsi Chromatograph (model DI-700) and a PDP-1173 (DEC) computer using a SP 2380 column (25 m × 0·32 mm) with a temperature programme of 180–220°C at a rate of 2·5°C/min.

^{13}C-Nuclear magnetic resonance (^{13}C-NMR) spectra were obtained on either AM 300 or AM 400 Bruker Spectrometer, both equipped with a process controller, an Aspect 3000 computer and a variable temperature system. The galactomannan samples were dissolved in D_2O (20 mg/ml) at 75°C. After complete dissolution, the solutions were sonicated for 10 min, centrifuged and poured into NMR tubes. Spectra were recorded at 80°C under conditions of inverse gated decoupling. Peak integrals were performed using the Bruker software and assignments were made with reference to results already published (Noble & Taravel, 1987).

Average molecular weight and intrinsic viscosity measurements were performed on a Waters 150-C ALC/GPC with three detectors on line at 25°C (Tinland *et al.*, 1988), and for 1 g/l galactomannan solutions after filtration through 0·2 μm Millipore filters.

Films were formed as coatings on heated Teflon plates by slow evaporation of 0·5% (w/v) solution over 24 h. The films were cut into strips about 1·5 mm wide and 7 mm long, placed between clamps and were hung with a load of 10–20 g at 95% relative humidity (RH) for 5–7 days until elongation of about 250–450% was reached. Crystallinity of the stretched films was further enhanced by annealing in a sealed high pressure bomb at 100°C over aqueous $CuSO_4$ for 4 h. The resulting stretched films were mounted onto a 0·20 mm diameter collimator and exposed *in vacuo* to CuKα ($\lambda = 1{\cdot}5418$ Å) radiation from a Philips PW 4720 X-ray generator for 18–20 h, using a Warhus flat-film vacuum camera.

RESULTS AND DISCUSSION

Polysaccharide characterization

Upon complete hydrolysis, purified galactomannan samples showed the presence of D-galactose and D-mannose. Their ratios, as determined by gas–liquid chromatography (GLC) and ^{13}C-NMR, are reported in Table 1. The sugars were characterized with GC–MS and their *m/z* values found to be identical to those in the literature (Gupta & Grasdalen, 1988). The resonances of all ^{13}C-NMR nuclei were fully resolved and readily identified (Grasdalen & Painter, 1980; Noble & Taravel, 1987). The mannose/galactose ratios, calculated from the relative intensities of C-1 signals, were in good agreement with those obtained by chemical analysis (Table 1). The galactomannan from *Medicago sativa* was found to be identical to the previously reported lucerne gum (Song *et al.*, 1989).

The NMR spectra of the four samples displayed the same fundamental pattern characterizing galactomannan polysaccharides as reported in the literature for other seed gums (Grasdalen & Painter, 1980; Noble & Taravel, 1987). GPC results of 1 g/l solution are also presented in Table 1. Of these samples, *Cassia siamea*

Table 1. Preliminary analysis of seed galactomannans from *Trifolium alexandrium*, *Medicago sativa*, *Cassia siamea* and *Cassia saltiana*

Source	Mannose/galactose ratio GLC	Mannose/galactose ratio ^{13}C-NMR	Weight average molecular weight $\overline{M}_w$	Number average molecular weight $\overline{M}_n$	Intrinsic viscosity ml/g $[\mu]$
Trifolium alexandrium	1·23	1·12	$4{\cdot}63 \times 10^5$	$2{\cdot}98 \times 10^5$	224·0
Medicago sativa (lucerne)	1·16	1·04	$2{\cdot}88 \times 10^5$	$1{\cdot}35 \times 10^5$	216·0
Cassia siamea	2·55	2·46	$8{\cdot}41 \times 10^5$	$7{\cdot}66 \times 10^5$	1160·0
Cassia saltiana	2·29	2·20	$2{\cdot}88 \times 10^5$	$1{\cdot}33 \times 10^5$	393·0

had the highest molecular weight $\overline{M}_w$ of $8{\cdot}41 \times 10^5$ and an intrinsic water viscosity of 1160 ml/g. The molecular weight and intrinsic viscosity of the other samples ranged from 2·88 to $4{\cdot}63 \times 10^5$ and 216 to 393·0 ml/g, respectively. It is worth noting that, owing to a higher molecular weight, *C. siamea* could be stretched by 450%, whereas the other samples reached 250–300% before breaking. All samples showed a tendency to relax if not held at constant extension when stored at high humidity.

Diffraction patterns and crystallography

Typical diffraction patterns obtained in vacuum are shown in Fig. 1. The pattern obtained for *Medicago sativa* from India, is identical to the reported pattern of lucerne gum (Song *et al.*, 1989). The diffraction patterns of *Cassia* gums having degrees of substitution of 0·44 and 0·39, respectively, differ from the patterns of *Medicago sativa* (0·86) and *Trifolium alexandrium* (0·81) having more galactose. *Cassia saltiana* shows great similarity to *Cyamopsis tetragonolobus* or guar galactomannan (Palmer & Ballantyne, 1950; Winter *et al.*, 1984; Veluraja & Atkins, 1988), whereas *C. siamea* has more resemblance to tara gum or *Caesalpinia spinosa* (Marchessault *et al.*, 1979; Chien & Winter, 1985; Cairns *et al.*, 1986) having $a = 8{\cdot}91$ Å, $b = 24{\cdot}17$ Å and $c = 10{\cdot}46$ Å. *Cassia siamea*, whose intrinsic viscosity and molecular weight are the highest, gave the best chain stretching and ordering.

Measured and calculated interplanar spacings (*hkl* indices) are reported in Table 2. The lattice constants for the orthorhombic unit cells derived from these patterns and those reported for other galactomannans are presented in Table 3. The relationship between d_{020} spacing and Gal/Man ratio for these samples is shown in Fig. 2.

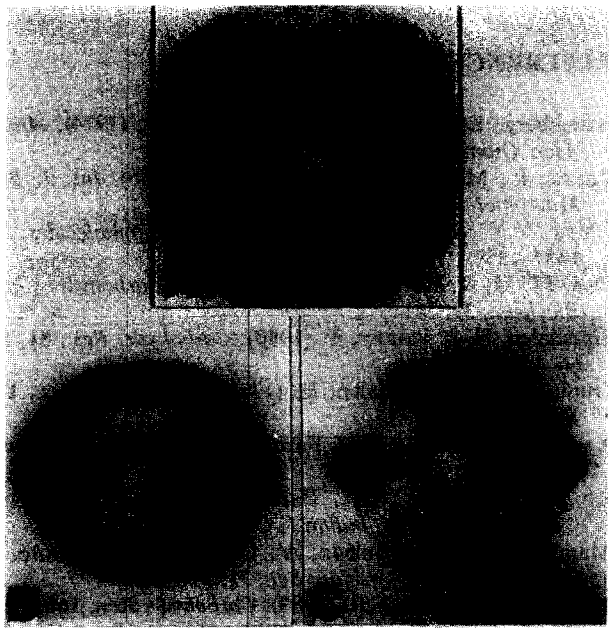

Fig. 1. X-ray diffraction pattern *in vacuo* at room temperature of (A) *Trifolium alexandrium*, (B) *Cassia saltiana* and (C) *Cassia siamea*.

Table 2. Interplanar *d* spacings (Å) for some seed galactomannans

hkl	*Trifolium alexandrium*	*Medicago sativa* (lucerne)	*Cassia siamea*	*Cassia saltiana*
020	15·40	15·33	12·40	12·38
001	10·27	10·24	10·30	10·30
100	—	—	9·00	8·99
011	9·74	9·71	—	—
110	8·65	8·64	8·46	8·45
120	7·78	7·76	7·28	7·27
111	—	—	6·53	6·53
040	7·70	7·76	6·20	6·19
130	6·78	6·75	—	—
121	6·20	6·18	—	—
140	5·86	5·83	5·10	5·09
002	5·13	5·12	5·15	5·15
022	—	—	4·75	4·75
112	4·42	4·40	—	—
102	—	—	4·47	—
220	4·33	4·31	4·23	4·22
230	4·13	4·12	—	—
132	4·09	4·08	—	—
042	—	—	3·96	3·99
240	3·89	3·88	3·64	—
152	3·61	3·60	—	—
241	—	—	3·43	—
003	3·44	3·41	—	—
113	3·18	3·17	—	—
040				
240	—	—	3·64	3·63
241			3·43	3·43

The results revealed relative invariability of the *a* and *c* dimensions of the unit cell, whereas the *b* dimension varied substantially in the different samples, ranging from 30·66 to 30·80 Å in the highly substituted gums but decreasing to 24·75 and 24·81 Å in *Cassia* samples. Chien and Winter (1985) have already mentioned the sensitivity of the *b* dimension to the degree of galactose substitution and have shown that it decreases with decreasing galactose content. This dimension is also sensitive to hydration (data not reported here) as described by Song *et al.* (1989) who found variations of more than 15% for samples maintained at constant RH values during X-ray experiments, compared to those recorded under vacuum (Winter *et al.*, 1984, 1987). According to these authors, for the entire range of galactose substitution, from 0% in mannan I to 93% in fenugreek polysaccharides, the structure is essentially the same and consists of an energetically preferred main-chain conformation together with a regular lateral association. Any gaps created by the absence of galactose substitution are expected to be filled with water at high humidities. The probable space group symmetry of the unit cell is $P2_12_12$ (Song *et al.*, 1989).

Table 3. Orthorhombic unit cell parameters (Å) for galactomannans

Source	Man/Gal	*a*		*b*		*c*	
		Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
Trifolium alexandrium	1·23	9·02	8·99	30·80	30·80	10·27	10·26
Medicago sativa (lucerne)	1·16	9·00	8·99	30·66	30·65	10·24	10·22
Cassia siamea	2·55	9·00	9·00	24·81	24·62	10·30	10·30
Cassia saltiana	2·29	8·99	9·01	24·75	24·62	10·30	10·30
Fenugreek[a]	1·08		8·94		29·50		10·27
Lucerne gum[a]	1·09		8·99		31·09		10·31
Tara[b]	2·56		8·91		24·17		10·46
Gleditsia Amorphoides[c]	3·00		8·8		24·4		10·3
Mannan I[a]			8·92		7·21		10·27
Mannan II[d]			9·01		16·73		10·40

[a]Obs, observed; Calc, calculated. From Song *et al.*, 1989.
[b]From Chien & Winter, 1985.
[c]From Aisemberg *et al.*, 1974.
[d]From Yui *et al.*, 1992.

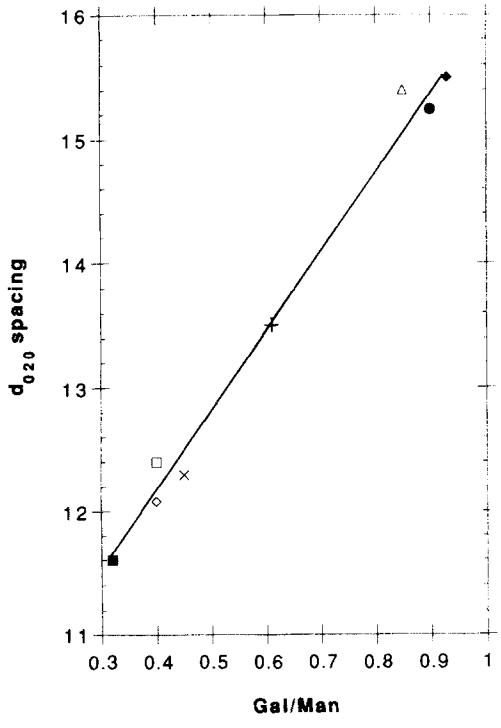

Fig. 2. Relationship between the observed X-ray d_{020} spacing (Å) and the galactose/mannose ratio for the galactomannans studied from *Medicago sativa* (lucerne) (●), *Trifolium alexandrium* (△), *Cassia saltiana* (×) and *Cassia siamea* (□). These data are compared to others already reported from locust-bean (■), tara gum (◇), guar (+) and fenugreek (◆) (Marchessault *et al.*, 1979; Song *et al.*, 1989).

The Indian galactomannans described in this note show structural characteristics which are consistent with the general accumulated knowledge concerning galactomannans. They are readily available and could offer the same industrial potential as those commonly used, such as guar and carob galactomannans. In particular, they could display interesting synergistic properties when mixed with other polysaccharides, such as agarose, carrageenan or xanthan. Of the four samples that have been studied, *Cassia siamea* seems the most promising, as it has the highest molecular weight and is, therefore, the most viscous.

ACKNOWLEDGEMENTS

The authors thank Mr Jean Mazet for polysaccharide characterization. One of us (V.P. K.) thanks the French Ministry of Foreign Affairs for a 12 month fellowship.

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