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Note

Structure of a galactomannan from the seeds of *Cassia angustifolia* Vahl

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Abstract

Cassia angustifolia Vahl (family: Caesalpiniaceae) is a fast growing and spreading Indian shrub of which seeds, pods and leaves are extensively used for pharmaceutical applications. The seeds have been found to be an alternative source of commercial gums. The structural aspects of the galactomannans have been determined for a better understanding of its properties. The purified seed galactomannan contains mannose:galactose in a ratio of 2.90. The average molecular weight ($\bar{M}_w$) is 9.66×10^4 and the intrinsic viscosity (η) is 209 mL/g. Methylation analysis, periodate oxidation, Smith degradation and ^{13}C NMR studies confirm that the gum has the basic structure of legume galactomannans with a main chain of (1 → 4)-linked β-D-mannopyranosyl units to which single α-(1 → 6)-D-linked galactopyranosyl units are attached through block pattern. © 2001 Published by Elsevier Science Ltd.

Keywords: Cassia angustifolia; Galactomannan; Galactopyranose; Mannopyranose

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Seed galactomannans[1,2] are important agrochemicals used in various industries[3] worldwide. The growing industrial utility of these gums or modified derivatives in the field of paper, textile, petroleum recovery and pharmaceutical industries, has resulted in an impetus in India for intensified research on new sources having varying galactose–mannose ratios and fine structures. They are non-toxic, inexpensive and eco-friendly and have been considered as GRAS (generally recognised as safe).[4,5] Our R&D studies show that *Cassia angustifolia* seed gum possesses the potential to become a new source of commercial gum and can be used as an additive for the preparation of pharmaceutical formulations.[6] Structural study is a basic requirement for understanding the gum properties, particularly the rheological, gelling and interaction behaviours, which are considered to be directly related to the structural features. Keeping this in view, the structural studies of the seed gum has been carried out and is presented in the present note. This is the first report on the structural elucidation of a galactomannan isolated from the seed endosperm of *C. angustifolia*, however Mullar and Kraus[12] have reported the structure of a water-soluble polysaccharide extracted from the leaves of the plant, consisting of L-rhamnose, L-arabinose, D-galactose and D-galacturonic acid.

C. angustifolia Vahl syn. *C. acutifolia* Delite; syn. *C. senna* Linn. var. Senna is a

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branching shrub,[7] with a height up to 1.8 m, found in abundance throughout south India and other parts of the country. Some taxonomists treat it as *Senna alexandrina* Miller; syn. *C. angustifolia* Vahl; *C. senna* Linn.[8] The whole plant is medicinally important, mainly for its cathartic property, and used as laxative.[9–11] The seeds are creamish to brown in colour, obovate–oblong, dicotyledonous and medium sized (3–4 × 1.5–2.0 × 4–5 mm; wt. of 100 seeds ~ 2.65g). They contain endosperm, 48–52%; crude protein, 10.6%; pentosan, 5.9%; total ash, 4.1%; water-soluble gum, 34%; alcohol extractive, 9–10%; ether extractive, 5.8% and hexane extractive, 6.1%. An analysis of seed parts is given in Table 1. The seeds have been found to be a potential source of commercial gums as they contain about 50% of the endosperm, which are easily separable by mechanical means. The standard specifications of the gum, along with ISI and US codes for guar gum, are presented in Table 2.

The crude gum was purified by the usual procedure of fractional precipitation with ethanol and barium complexing. The results of intrinsic viscosity and average-molecular weights of the gum are presented in Table 3. Constituent sugars were identified by GC–MS and their m/z values have been found identical with literature.[13] The ratio of constituent sugars was determined by HPLC and GLC

Table 1
Chemical analysis of the seed components of *C. angustifolia*

Seed parts	Protein (%)	Moisture (%)	Ether extract (%)	Ash (%)	Crude fibre (%)	Water-soluble carbohydrates
Seed coat (20–24%)	12.1	5.2	2.4	8.7	20.7	pentose
Endosperm (48–52%)	4.2	11.2	0.7	1.15	0.8	galactomannan
Germ (28–32%)	41.6	7.8	9.1	4.5	11.8	pentose, glucose

Table 2
Standard specifications of food grade *C. angustifolia* and guar gum

Components	Typical composition of *C. angustifolia* gum (%)	ISI code[a] for guar gum (%)	Food chemical specification (USA) for guar gum (%)
Galactomannan	82	75.0 (min)	66.0 (min)
Acid insoluble (crude fibre)	0.8	7.0 (max)	7.0 (max)
Protein	4.2	5.0 (max)	10.0 (max)
Moisture	11.2	13.0 (max)	
Fat	0.54		
Ash	1.2		
Arsenic			3.0 ppm
Heavy metal like lead			10.0 ppm
Starch	nil		passes iodine test
pH of aq solution	6–7	5.5–8.0	
Viscosity (%)			
(1)	1175 cP	2500 cP	
(2)	9400 cP		
(3)	49 250 cP		

[a] Indian specification of guar gum, ISI, New Delhi, 15: 3936-1981, Food Chemical Code (3rd ed.) 1981, National Academy Press, Washington, DC, 191.

Table 3
Physico-chemical analysis of *C. angustifolia* seed galactomannan

Average molecular weight	Polydispersity	d*n*/d*c*	Intrinsic viscosity (mL/g)
$\bar{M}_n = 8.68 \times 10^3$ $\bar{M}_w = 9.66 \times 10^4$ $\bar{M}_z = 3.28 \times 10^5$	$\bar{M}_w/\bar{M}_n = 11.13$	0.15	209 $k' = 0.86$

Table 4
Sugar analysis of *C. angustifolia* seed galactomannan

Sugars	Sugar ratio				*m*/*z* (GC–MS)
	HPLC	GLC[a]	^{13}C NMR	^{1}H NMR	
D-Galactose	1.00	1.00	1.00	1.00	43, 73, 107, 145, 217, 289
D-Mannose	2.90	2.92	2.63	2.82	43, 73, 107, 145, 217, 289

[a] As corresponding alditol acetate derivative.

(through the corresponding alditol acetate derivatives). The polysaccharide has been characterized as a pure galactomannan comprising D-galactopyranosyl and D-mannopyranosyl units (Table 4). The ratio of sugars has also been confirmed by ^{1}H and ^{13}C NMR spectroscopy via the relative areas of the anomeric galactopyranosyl and mannopyranosyl unit signals (substituted and non-substituted) (Table 4).

The purified galactomannan was converted into its fully methylated derivative using two successive Hakomori methylations[14] followed by two subsequent Purdie methylations.[15] Hydrolysis of the permethylated polysaccharide yielded three compounds, viz. 2,3,4,6-tetra-*O*-methyl-D-galactose (0.96 mol), 2,3,6-tri-*O*-methyl-D-mannose (1.97 mol) and 2,3-di-*O*-methyl-D-mannose (1.00 mol). On the basis of the methylation analysis, it can be concluded that the gum possesses the basic structure of a galactomannan having a main-chain of (1 → 4)-linked mannopyranosyl units with single side-chains of galactopyranosyl units attached to the main chain through (1 → 6) linkages. In this case, on average, about 35% of the mannopyranosyl units are branched. By methylation analysis, the galactose:mannose ratio was found to be 1.0:3.1, which is in close agreement with the results of chemical analysis. The anomeric configurations were also determined by the chromium trioxide oxidation method,[16] which indicated the presence of β-D-mannopyranosyl units (oxidised more rapidly) and α-D-galactopyranosyl units.

The gum consumed 1.21 mol of periodate per hexosyl unit, with concomitant liberation of 0.22 mol of formic acid per hexosyl unit. Completion of the periodate reaction with reference to uptake of periodate required 7 days, as the reaction was carried out at 4 °C to avoid overoxidation. Moreover, the oxidation of mannopyranosyl units adjacent to oxidised 6-*O*-substituted mannosyl units is slow owing to hemiacetal formation.[17,18] The periodate oxidation results are in good agreement with the theoretical values of the proposed galactomannan structure based on methylation data. Periodate degradation of the polysaccharide yielded glycerol and erythritol in a molar ratio of 1.00:2.95. None of the monomer units survived periodate oxidation. These results further support the proposed structure of the galactomannan.

Resonances of the anomeric protons in ^{1}H NMR spectroscopy are well separated and identified. The doublet at δ 5.4 (H-1 of D-galactopyranosyl units) and the singlet at δ 5.1 (β-D-mannopyranosyl units) indicate that the D-galactopyranosyl and D-mannopyranosyl units in the polymer could have the expected $^{4}C_1$ conformation. Structural aspects of the galactomannan were also analysed by

Table 5
Assignment of peaks in the ^{13}C NMR spectrum of *C. angustifolia* seed galactomannan

Types of unit	Chemical shifts (δ, ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Galactopyranosyl	99.67	69.38	70.44	70.24	71.99	62.03
β-D-Mannopyranosyl unbranched at O-6	100.88	70.93	72.37	77.20 77.44	75.89	61.53
β-D-Mannopyranosyl branched at O-6	100.74	70.93	72.37	77.44 77.67	74.29	67.62 67.48 67.21

^{13}C NMR spectroscopy. The gum forms viscous solutions and successful spectroscopy was possible only after diminishing the viscosity by sonication. Resonances of all carbon atoms are fully resolved and readily identified. Spectra are in close agreement with those reported for other galactomannans[19–21] (Table 5). The spectrum shows the signals at δ 99.67 (anomeric carbon atom of galactopyranosyl units) and at δ 100.88 and 100.74 (anomeric carbon atoms of unbranched and branched mannopyranosyl units). The relative peak areas were 8.349:21.960 (1.00:2.63), which is in reasonable agreement with the results of chemical analysis. During the ^{13}C NMR studies, a comparative low proportion of mannose–galactose is observed which may be due to an incomplete dissolution of some portions of the galactomannan in deuterium oxide and the presence of aggregation at the concentration used. Resonances of C-2, C-3 and C-5 of galactopyranosyl and mannopyranosyl units are well-resolved lines and readily identified. C-4 of mannopyranosyl units produced three resolved signals corresponding to branching patterns A, B and C (Fig. 1). Signals in the triplet result from the three nearest neighbour probabilities of mannopyranosyl units in the main chains.[19] In the spectrum, peaks B and C are more prominent (about 90%) than the peak A (about 10%), suggesting larger proportions of the regions having two contiguous mannopyranosyl units with single or non-substituted galactopyranosyl units. Manzi et al.,[22] compared the C-6 resonances, which were clearly resolved in the spectra of depolymerised galactomannans from *Gleditsia triacanthos* having different Man:Gal ratios. They suggested that the peak at the lowest field originated from the C-6 resonance of the intermediate unit from groups of three contiguous substituted D-mannosyl units as in triad (I). The signal at higher field is due to blocks of three contiguous D-mannosyl units, where only the intermediate residue is substituted — triad (III) — and the last peak represents the superimposition of signals from triad (II), where only two contiguous units are substituted. The observations are consistent with the arrangements having the dominance of triad II over other triads (I and III) suggesting attachment of side chains in block pattern.

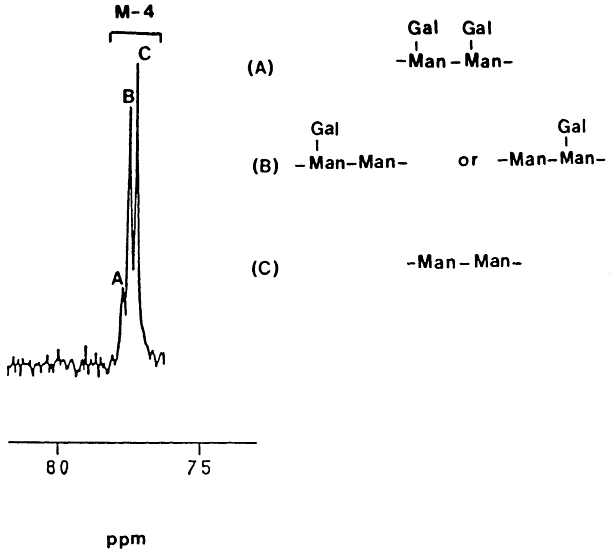

Fig. 1. ^{13}C NMR spectral region of C-4 (mannose); A, B and C are probable diad interpretation.

1. Experimental

Isolation and purification.—The seeds of *C. angustifolia* were collected from South India during May and June. The seeds were roasted, and the endosperm was separated from the germ and hull by a dry milling process using various mixers, grinders and sieves and finally powdering to 100 mesh with a hammer mill (yield, 40.0%). The crude gum was purified by EtOH precipitation and Ba^{2+} complexing methods. The gum was finally purified by dissolving the polysaccharide in water (1g/L; w/v) and dialysing for 40 h in running distilled water, followed by concentration, precipitation with EtOH, filtration and drying in vacuum at 40–50 °C.

Molecular weight determination.—Average molecular weights and polydispersity of the purified galactomannan were determined according to a previously described method.[21]

Constituent sugar analysis.—The mannose:galactose ratio was determined after complete hydrolysis of the galactomannan with 1 M H_2SO_4 at 100 °C for 18 h (Waters HPLC, Waters Carbohydrate column No. P/N 85188). For GLC analysis, the galactomannan was hydrolysed with 1 M trifluoroacetic acid (4 h; 100 °C) followed by reduction and acetylation. The corresponding alditol acetate derivatives of the sugars were analysed by GLC and GC–MS according to previously described methods.[21]

Methylation analysis.—For methylation analysis, the gum was suspended in freshly distilled Me_2SO and extensively stirred for 22 h at rt, followed by mild sonication for 20 min and stirring for another 5 h. The partially dispersed product was methylated according to the Hakomori method.[14] The product was again subjected to Hakomori methylation, followed by two successive Purdie methylations.[15] The completely methylated polysaccharide showed no IR–OH absorption (3600–3400 cm^{-1}). The methylated polysaccharide was boiled under reflux with HCOOH. The resulting methylated sugars were converted to the corresponding alditol acetate derivatives and analysed by GLC and GC–MS.[21]

Periodate oxidation.—The polysaccharide (90 mg) was dissolved in water (25 mL) and the solution was cooled to 0 °C. A cold solution of sodium metaperiodate (0.15 M, 30 mL) was added to the solution and volume was made up to 100 mL. The reaction was conducted at 4 °C and the amounts of periodate consumed and formic acid liberated, were estimated at different time intervals. The periodate oxidation was completed in 165 h.

Smith type degradation.—The polysaccharide (1 g) was oxidised with sodium metaperiodate as mentioned above. After removing the excess of periodate, the solution was reduced with $NaBH_4$ (0.9 g) at rt for 20 h. The solution was neutralised (HOAc), deionised and evaporated to dryness. After removing boric acid by repeated evaporations with MeOH, the residue was hydrolysed with 1 N H_2SO_4 (100 mL) for 15 h at 95 °C. The hydrosylate was neutralised ($BaCO_3$), filtered, deionised and evaporated. PC using the solvent system (4:1:5; upper layer) *n*-BuOH–EtOH–water and *p*-anisidine phosphate as spray reagent revealed the presence of glycerol and erythritol (characterised by chromatography with standard glycols). Glycerol and erythritol were quantified by the chromotropic acid method.[23]

NMR studies.—The ^{13}C NMR spectra were done by dissolving 20 mg/mL galactomannan in D_2O at 70 °C with continuous stirring for 6 h, followed by mild sonication for 10 min. The solution was centrifuged and transferred to a 5-mm NMR tube. Spectra and assignments were obtained according to Ref. 21 and 20, respectively published. Proton spectra were obtained according to Ref. 21.

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