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Note

N.m.r. studies of composition and side-chain arrangement in *Sesbania aegyptiaca* seed galactomannan*

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Bhattacharyya *et al.*[1] examined the structure of a water-soluble galactomannan isolated from *Sesbania aegyptiaca* seeds by chemical methods and suggested that it contains a main chain in which no groups of two contiguous unsubstituted D-mannopyranosyl units occur. However, methylation analysis, the primary structural technique used, is dependent on complete methylation and subsequent total hydrolysis of the methylated polymer, and provides only information on the mole percentages of the various chain and branch-point units; it gives no information on the relative position of these residues in the polymer. Nondestructive n.m.r. spectroscopy provides a convenient technique complementary to methylation–fragmentation analysis. It can disprove or confirm previous structural findings and provide new, independent structural information, *i.e.*, information about nearest-neighbor sequences and anomeric configurations[2]. We have used n.m.r. spectroscopy to obtain information on the fine structure of the galactomannan of *Sesbania aegyptiaca* seed.

The resonances of the anomeric protons in the ^{1}H-n.m.r. spectrum (see Fig. 1) are well separated, and their identification is evident from the known monomeric composition of the polysaccharide (Gal:Man = 1:1.67)[1]. The spectrum contains a signal at 4.8 p.p.m. ($J_{1,2}$ ~1.0 Hz) of the anomeric proton (H-1) of the D-mannopyranosyl units which, accordingly, must have the β-D configuration and be in the expected $^4C_1$ conformation[2,3]. The doublet at 5.0 p.p.m. ($J_{1,2}$ ~3.2 Hz) is assigned to H-1 of the galactopyranosyl units which, therefore, must have the expected α-D configuration and $^4C_1$ conformation[2,3].

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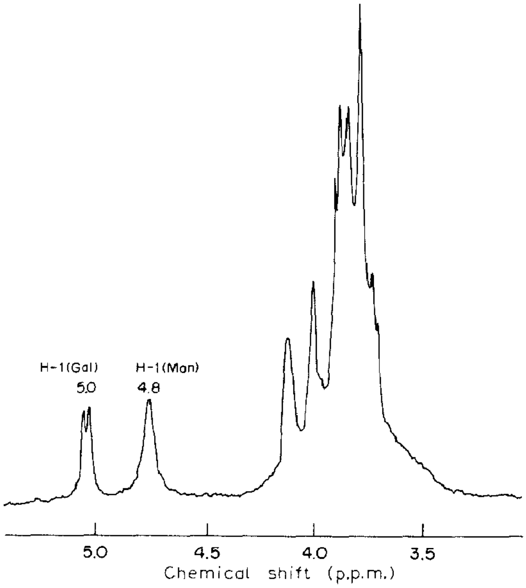

Fig. 1. ^{1}H-N.m.r. spectrum (100 MHz) of a solution (2 mg/0.4 mL) of *S. aegyptiaca* seed galactomannan in D_2O at 90° and pD 7.

In the ^{13}C-n.m.r. spectrum (see Fig. 2), the separated lines are in accord with those reported for guaran[2] and carob (locust bean) gum[2]. Their chemical shifts are recorded in Table I. Three types of structural unit can be clearly differentiated and identified. These units are (*i*) the α-D-galactopyranosyl nonreducing end-units, (*ii*) the otherwise unsubstituted (1→4)-linked β-D-mannopyranosyl units of the mannan backbone, and (*iii*) the O-6-substituted, (1→4)-linked β-D-mannopyranosyl units of the mannan backbone. The spectral regions of the anomeric carbon atoms (101.8 and 103 p.p.m.) and the methylene carbon atoms (63.6 and

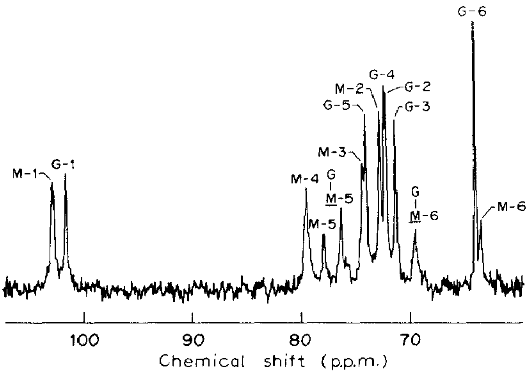

Fig. 2. ^{13}C-N.m.r. spectrum (25 MHz) of a solution (25 mg/0.4 mL) of *S. aegyptiaca* seed galactomannan in D_2O at 95° and pD 7.

TABLE I

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

Unit	*Chemical shift*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-D-Galactopyranosyl	101.8	72.3[b]	71.4	72.4[b]	74.1	64.1
4-*O*-Substituted β-D-mannopyranosyl (unbranched at O-6)	103.0	72.9	74.4	79.3[c] 79.6[d]	78.0	63.6
4,6-Di-*O*-substituted β-D-mannopyranosyl (branched at O-6)	103.0	72.9	74.4	79.6[c] 79.9[d]	76.3	69.6

[a] Shifts (p.p.m.) downfield from internal sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. [b] These assignments may have to be interchanged. [c] When the preceding D-mannosyl unit is unbranched. [d] When the preceding D-mannosyl unit is branched. Apart from the major resonance at 79.9 p.p.m., the values of the chemical shifts for C-4 of D-mannopyranosyl units were taken from ref. 2.

64.1 p.p.m.) are well documented[2,4]. Three resonances for the C-4 signal of the (1→4)-linked β-D-mannopyranosyl units were noted. The same phenomenon, observed in the spectrum of guaran, was attributed to sequence-related heterogeneity, explained by assuming that the resonance is sensitive to whether its nearest neighbor on the nonreducing side is a branch-point unit, and it was used to determine the nearest-neighbor probabilities in the central chain of galactomannans[2]. According to the concept, the absorption at the lowest field (79.9 p.p.m.) originates from sets of two contiguous, substituted D-mannopyranosyl units; the intermediate peak (79.6 p.p.m.) represents the superposition of signals originating from diads in which only one of the two mannosyl units is substituted, and the peak at high field (79.3 p.p.m.) is assigned to unsubstituted D-mannopyranosyl units that are adjacent to other units of the same kind. Because the substituted D-mannosyl units are branched at O-6, the positions of their C-6 (69.6 p.p.m.) and C-5 (76.3 p.p.m.) resonances are shifted relative to those of the corresponding resonances of unbranched D-mannopyranosyl units, namely, 6 p.p.m. downfield and 1.7 p.p.m. upfield, respectively. They are, therefore, readily identified and are in agreement with literature values[2].

Expansion of the resonances in the C-4(Man) region is given in Fig. 3. The peak at high field (designated III) is assigned to contiguous unsubstituted D-mannosyl units. The peak at the lowest field (I) is assigned to contiguous D-mannosyl units substituted with D-galactosyl units. The intermediate peak (II) is believed to represent a superposition of signals originating from diads in which only one of the two D-mannosyl units is substituted.

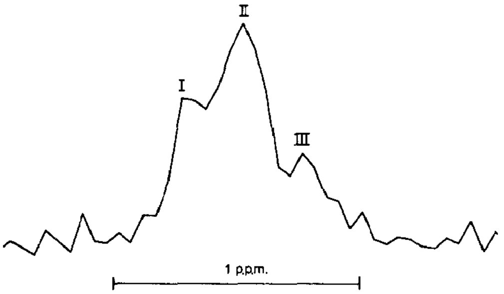

Fig. 3. C-4(Man) spectral region. [Interpretation (the unit involved is underlined): (I) $\begin{array}{ccc} \text{Gal} & & \text{Gal} \\ | & & | \\ \text{--Man} & \text{---} & \underline{\text{Man}}\text{--} \end{array}$ (II) $\begin{array}{c} \text{Gal} \\ | \\ \text{--Man-}\underline{\text{Man}}\text{--} \end{array}$ and $\begin{array}{c} \text{Gal} \\ | \\ \text{--Man-}\underline{\text{Man}} \end{array}$ (III) --Man-$\underline{\text{Man}}$--].

The diad frequencies for *Sesbania aegyptiaca* seed galactomannan, as obtained by ^{13}C-n.m.r. spectroscopy, are summarized in Table II, together with those calculated for a random distribution given by $F_{11} = (F_1)^2$, $F_{22} = (1 - F_1)^2$, and $F_{12} = F_{21} = F_1(1 - F_1)$. F_1 is identical with the Gal:Man ratio measured by ^{13}C-n.m.r. spectroscopy. The good agreement between the two sets of values permits the discarding of structures having a strictly alternating arrangement of branched and unbranched D-mannosyl units, as well as structures in which a block of branched D-mannosyl units is contiguous to a block of unsubstituted D-mannosyl units. The estimated diad frequencies suggest that *Sesbania aegyptiaca* seed galactomannan has an almost random arrangement of D-galactosyl groups. Using the same n.m.r. techniques, a random arrangement of D-galactosyl units along the mannan chain was proposed for the galactomannans from seeds of guar[2], carob (locust bean)[2], clover[2], lucerne[2], and *Gleditsia triacanthos*[5]. However, as pointed out by Painter[6], the presence of more-complex kinds of sequences is not disallowed.

TABLE II

COMPOSITIONS AND DIAD FREQUENCIES[a] FOR *S. aegyptiaca* SEED GALACTOMANNAN

Method[b]	*Gal:Man ratio*	*Diad frequencies*[a]		
		$F_{1,1}$	$F_{1,2} = F_{2,1}$	$F_{2,2}$
A	0.65			
B	0.62	0.36	0.24	0.16
C	0.62			
(D)	0.62	(0.38)	(0.24)	(0.14)

[a] The subscripts 1 and 2 refer to branched and unbranched D-mannosyl units, respectively. [b] A, ^{1}H-n.m.r.; B, ^{13}C-n.m.r.; C, periodate oxidation; (D), calculated for a random distribution from the Gal:Man ratio measured by method B.

Measurement of monomeric composition (Gal:Man ratio). — The relative areas of the signals for H-1(Gal) and H-1(Man) (see Fig. 1) yielded, directly, mole fractions of the two monomers (see Table II). The relative abundance of the two monomers was also obtained directly from the relative peak areas of corresponding ^{13}C-n.m.r. signals. In addition, reliable values of the Gal:Man molar ratio are provided by the sequentially uplifted C-5(Man) and C-6(Man) resonances. The ratio is given by the intensity of the shifted peaks over the total intensity of these carbon atom signals. Previously, it was shown that the Gal:Man ratio is also given by the sum of the diad frequencies (F_{11} + F_{12}) obtained from the sequentially split C-4(Man) resonances[2]. The Gal:Man ratio from ^{13}C-n.m.r. spectra, given in Table II, represents an average value (four analyses). The agreement with the results obtained by periodate oxidation[1], which is included for comparison, is good. However, the value given by ^{1}H-n.m.r. spectroscopy is significantly higher. This seems to be typical of samples having a high molecular weight, especially at high concentration. It is probably caused by selective aggregation of regions of the chain in which D-mannosyl units are unbranched[2].

EXPERIMENTAL

Purified *Sesbania aegyptiaca* seed galactomannan was prepared by the method of Bhattacharyya *et al.*[1]. The homogeneity of the polymer was established by moving-boundary electrophoresis (LKB modal electrophoresis apparatus) in 0.2M Tris·HCl buffer (pH 8.5) at 12 V and 5 mA for 5 h. The free-boundary migration, recorded with a Schlieren optical system, showed a single sharp symmetrical peak in both the descending and the ascending arms[8]. All n.m.r. experiments were done in the Fourier-transform mode with a JEOL FX100 spectrometer. The deuterium resonance was used as a field-frequency lock. ^{13}C-N.m.r. spectra were acquired by using 8000 data points and a spectral width of 5 kHz. Free-induction decay was accumulated with a 75° pulse and a repetition time of 0.8 s. Spectra in which the n.O. enhancements were removed were also measured, in order to ensure that relative peak areas represented relative abundances. A probe temperature of 95° was used to diminish viscosity and, thereby, line-width. Peak areas were measured by planimetry. The C-4 signal of a D-mannopyranosyl unit was reconstructed in a computer by superposition of three Lorentzian lines of equal width.

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