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Determination of Galactomannan by ^{1}HNMR & ^{13}CNMR Method

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Random distribution of D-galactose rsidue in side chains of Hibiscus cannabinus mannan has been confirmed by NMR analysis.

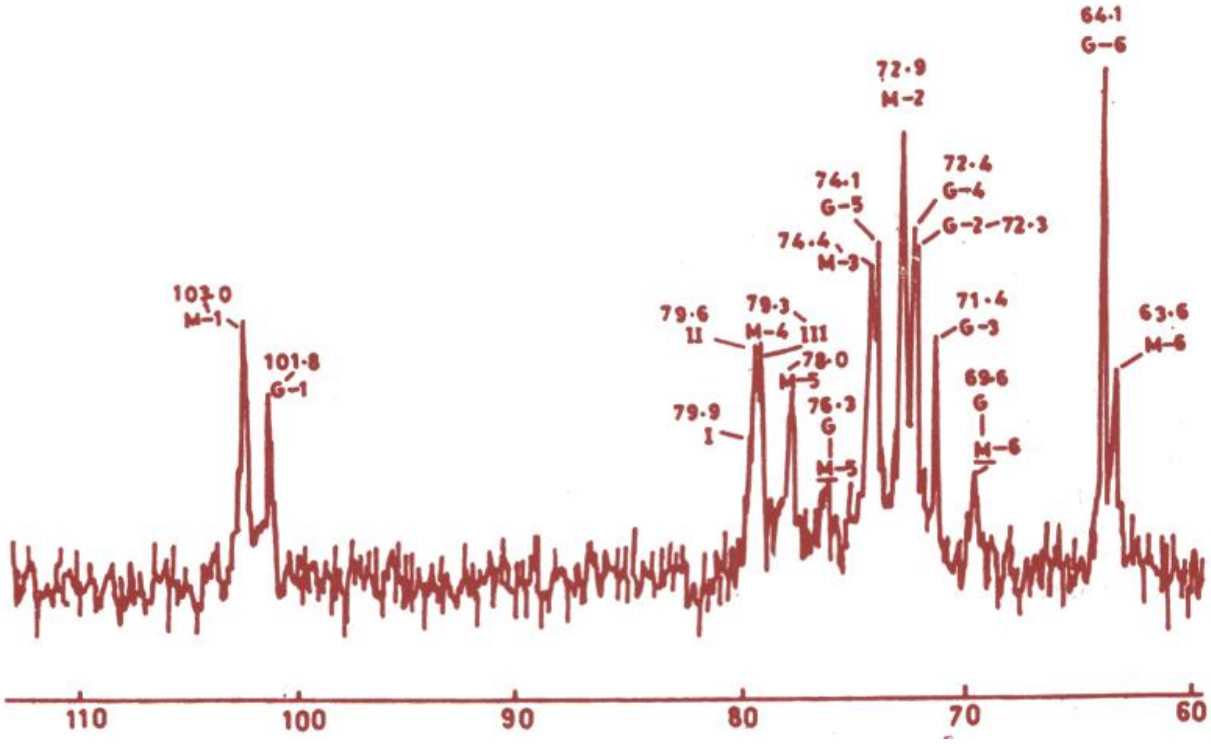
Purified polysaccharide isolated from H. cannabinus seeds on hydrolysis with M trifluoroacetic acid (4h at 100^{0}c), using paper chromatography and solvent n-butanol; Isopropanol : water (11:6:3) as solvent revealed the presence of galactose : Mannose in the ratio of 1:3. The structure estabished by usual chemical method. Investigation has been done by ^{1}H and 13C NMR spectra of galactomannon. Present structure determination based on complete assignments for protons and carbons as below:

The ^{1}H NMR spectrum showed, in the anomeric region, two absorptions. The signals at 4.9 ppm (J_1 ~ 2.9 Hz) and 4.6 ppm (J1,2~1.0 Hz) are assigned to H-1 of α-D-galactopyranosyl and β-D-mannopyranosyl units, respectively[2] which, therefore, should be in the expected $^{4}C_1$ conformation[2]. The integration of the anomeric proton resonances indicated a 1:3 ratio of galactose to mannose in the polymer.

In the ^{13}C NMR spectrum all the different carbon lies are well separated and are in accord with those reported for gum[3]. Their chemical shifts are in good agreement with those reported in the literature[3-5]. The spectrum clearly differentiated the three structural units of the polymer namely, 6-0-substituted and unsubstituted internal (1⟶4) linked β-D-mannopyranosyl units of the backbone and terminal (non-reducing) α-D-galactopyranosyl units. Integration of the anomeric carbon resonances at 101.8 and 103.0 ppm indicated a D-galcto—D-mannan molar ratio of 1.0:3. The peaks at 63.6 and 64.1 ppm were observed for the methylene carbon atoms[3 5]. Three resonances in the C-4 (Man) region were observed. **Accordingly, Figure shows** an expansion of the resonances in the C-4 (Man) region. The peak at the lowest field 79.9 ppm (designated I) originates from groups of two adjoining D-mannosyl units substituted with D-galactosyl units. The middle peak at 79.6 ppm (designated II) represents the superposition of signals originating from diads in which only one of the two mannose residues is substituted, and the peak at high field 79.3 ppm (designated III) is assigned to continuous unsubstituted D-mannosyl units. Another type of unit such as 6-0-substituted, (1⟶4) linked β-D-mannosyl internal residues of the central chain, could be identified, and in this case the positions of the C-6 (69.6 ppm) and C-5 (76.3 ppm) resonances are shifted, relative to the corresponding resonances of unbranched D-mannosyl residues (C-6 at 63.6 ppm and C-5 at 78.0 ppm) by 6.0 ppm downfield and 1.7 ppm upfield respectively. The assignment given for the carbon resonances of the D-galactose residues are well documented[3 5].

The diad frequencies for Hibiscus Cannabinus seeds galattomannan are given in Table I. The values of the diad frequencies as obtained' by ^{13}C NMR spectroscopy are in good agreement with those calculated for a random distribution using formula, $F_{11} = (F_1)^2$, $F_{22} = (1 - F_1)^2$, and $F_{12} = F_{21} = F_1 (1- F_1)$ where F_1, is the Gal : Man ratio measured by ^{13}C NMR spectroscopy and the subscripts 1 and 2 refer to branched and unbranched D-mannosyl units respectively[3,5]. The values of diad frequencies (Table I) discard not only the presence of regular arrangement of D-galactosyl residues in the side chains but also the presence of side chains in blocks. The good agreement in the two sets of the values of diad frequencies suggest the random distribution[3,5,6] of D-galactose residues in the side chains of polysaccharide structure.

Measurement of monomeric composition (*Gal : Man ratio*)—The relative areas of the signals (H-1 and C-1) of Gal and Man directly provided mole fractions of the two monomers (Table I). Gal : Man ratio as estimated by the sum of the diad frequencies ($F_{11} + F_{12}$) (Table 1) obtained from the sequentially split C-4 (Man) resonances[3,5], is also in good agreement with the estimates done by ^1H- and ^{13}C NMR spectroscopy, and periodate oxidation method[1] .



Experimental

The purified Hibiscus Cannabinus galactomannan was prepare by the method of Mukherjee et al.[1]. The purified polymer gave a single spot on paper electrophoresis. The ^1H NMR spectrum of the polymer was determined with a Bruker WH-400.13 spectrometer at 400.13 MHz and 348°K in D_2O (10 mg/1.0 ml) using TSP as reference standard. The ^{13}C NMR spectrum was recorded in the FT mode at 25 MHz with a FX100 spectro-meter; the sample (40 mg) was dissolved in D_20 (1 ml); temperature, 90°C; chemical shifts (in ppm) were expressed relative to internal sodium 3-(trimethyl-sily) propionate-d4. ^{13}C NMR spectrum was acquired by using 8000 data points and a spectral width of 5 KHz. The deuterium resonance was used as a field-frequency lock. Free-induction decay was accumulated with a 75° pulse and the repetition time of 0.8s. Spectrum 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 90°C was used to diminish viscosity and, thereby, line-width. Peak areas were measured by a planimeter.

Table 1 Diad frequencies for H.Cornna galactomannan

Gal : Man	**Diad frequencies**		
	$F_{1,1}$	**$F_{1,2} = F_{2,1}$**	**$F_{2,2}$**
0.36^a 0.36^b	011	0.24	02.41
0.36^c 0.36^d	0.15	0.25	(0.42)

(a)^{1}H NMR, (b, ^{13}CNMR (c) periodate oxidation[1]; (b) calculated for a random distribution from the Gal : Man ratio obtained by ^{13}C NMR spectroscopy.

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