

# **CHAPTER – VI**

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## **RESULTS AND DISCUSSION**

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In the enzymatic study of the polysaccharide the treatment of the polysaccharide with  $\alpha$ -D galactosidase hydrolysed the galactosyl residues and indicated the presence of galactose residues in the  $\alpha$  configuration. In  $^1\text{H}$  NMR spectrum (Fig.6.1) the doublet encountered at 5.15 ppm ( $J_{1,2} \approx 3.2$  Hz) and 4.8 ppm ( $J_{1-2} \approx 1.0$  Hz) are assigned to anomeric proton ( $\text{H}_1$ ) of  $\alpha$ -D-galactopyranosyl and  $\beta$ -D-mannopyranosyl units, respectively<sup>66</sup>. All the carbon lines in the  $^{13}\text{C}$  NMR spectrum of the polysaccharide (Fig.6.2) were resolved. Their chemical shifts are recorded in Table - 6.1. The resonances in the  $^{13}\text{C}$  NMR spectrum agree with those reported for guaran<sup>98</sup>, carob (Locust bean) gum<sup>98</sup> and *Sesbania aegyptiaca* seed galactomannan<sup>99</sup>. The spectrum, clearly, differentiated and identified three types of structural unit. These units are : i)  $\alpha$ -D-galactosyl non reducing end unit. ii) Unsubstituted (1  $\rightarrow$  4) linked  $\beta$ -D-mannosyl units of the mannan backbone and iii) O-6-substituted, (1-4)-linked  $\beta$ -D-mannosyl units of the mannan backbone. The anomeric carbon resonances at 101.88 and 103.01 ppm showed the presence of  $\alpha$ -D-galactopyranosyl and  $\beta$ -D-mannopyranosyl units, respectively, in the polymer<sup>98</sup>. The galactose mannose ratio obtained from  $^1\text{H}$  and  $^{13}\text{C}$  NMR, are in agreement with that of chemical analysis and illustrated in Table 6.2. The peaks at

63.65 and 69.61 ppm were observed for the methylene carbon atoms. Three resonances for the C-4 signal of the (1 → 4) linked β-D-mannosyl units were also noted in the <sup>13</sup>C NMR spectrum. Fig.6.3 shows an expansion of the resonances in the C-4 (man) region. The same phenomenon observed in the <sup>13</sup>C NMR spectrum of Guarana<sup>98</sup> was attributed to sequence related heterogeneity, explained by assuming that the resonance is sensitive to whether its nearest neighbour on the non reducing side is a branch point unit, and it was used to determine the nearest neighbour probabilities in the central chain of the galactomannan<sup>98</sup>. According to the concept the peak at the lowest field 79.89 ppm (designated I) originates from groups of two adjoining D-mannosyl units substituted with D-galactosyl units. The intermediate peak (II) at 79.67 ppm 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 79.34 ppm (designated III) is assigned to continuous unsubstituted D-mannosyl units.

The diad frequencies for *Sesbania grandiflora* seed galactomannan, as obtained by <sup>13</sup>C NMR spectroscopy are also illustrated in Table II, together with those calculated for a random distribution using formula :

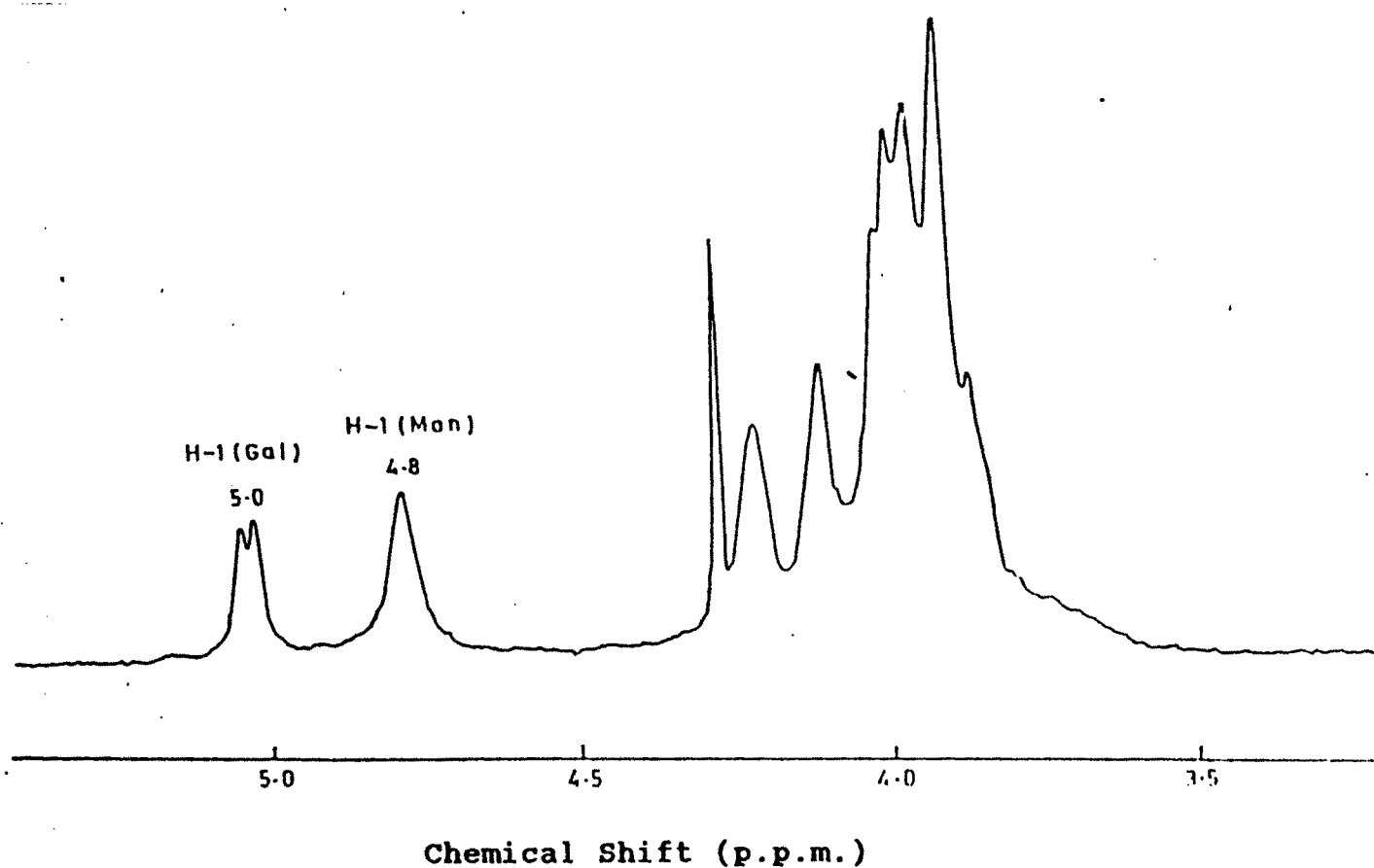
$$F_{1,1} = F_1^2 \quad F_{2,2} = (1 - F_1)^2$$

$$F_{1,2} = F_{2,1} = F_1(1 - F_1)$$

where  $F_1$  is identical with the Gal : man ratio measured by  $^{13}\text{C}$  NMR spectroscopy and the subscripts 1 and 2 refer to branched and unbranched D-mannosyl units, respectively. The good agreement between the two sets of values of diad frequencies permits the discarding of structures having regular arrangement of D-galactosyl residues in the side chains as well as structures in which the side chains of D-galactosyl residues occur in blocks. The values of diad frequencies suggest the random distribution of D-galactose residues in the side chains of *Sesbania grandiflora* seed polysaccharide structure, as is found in the structure of galactomannans from the seeds of guaran and carb (locust bean) gum<sup>98</sup>, *S.aegyptiaca*<sup>99</sup> and *cassia accidentalis*<sup>100</sup> using the same NMR techniques.

#### 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 two monomer's (Table 6.2) Gal : Man ratio as estimated by the sum of the diad frequencies ( $F_{1,1} + F_{1,2}$ ) (Table II) obtained from the sequentially split C-4 (Man) resonances<sup>98,99</sup> is also in good agreement with the estimates done by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy and periodate oxidation method<sup>28</sup>.



**Fig.6.1 :**  $^1\text{H}$  NMR Spectrum (MHz) of a solution (2mg/0.4ml) of *S. grandiflora* seed galactomannan in  $\text{D}_2\text{O}$  at  $90^\circ\text{C}$ .

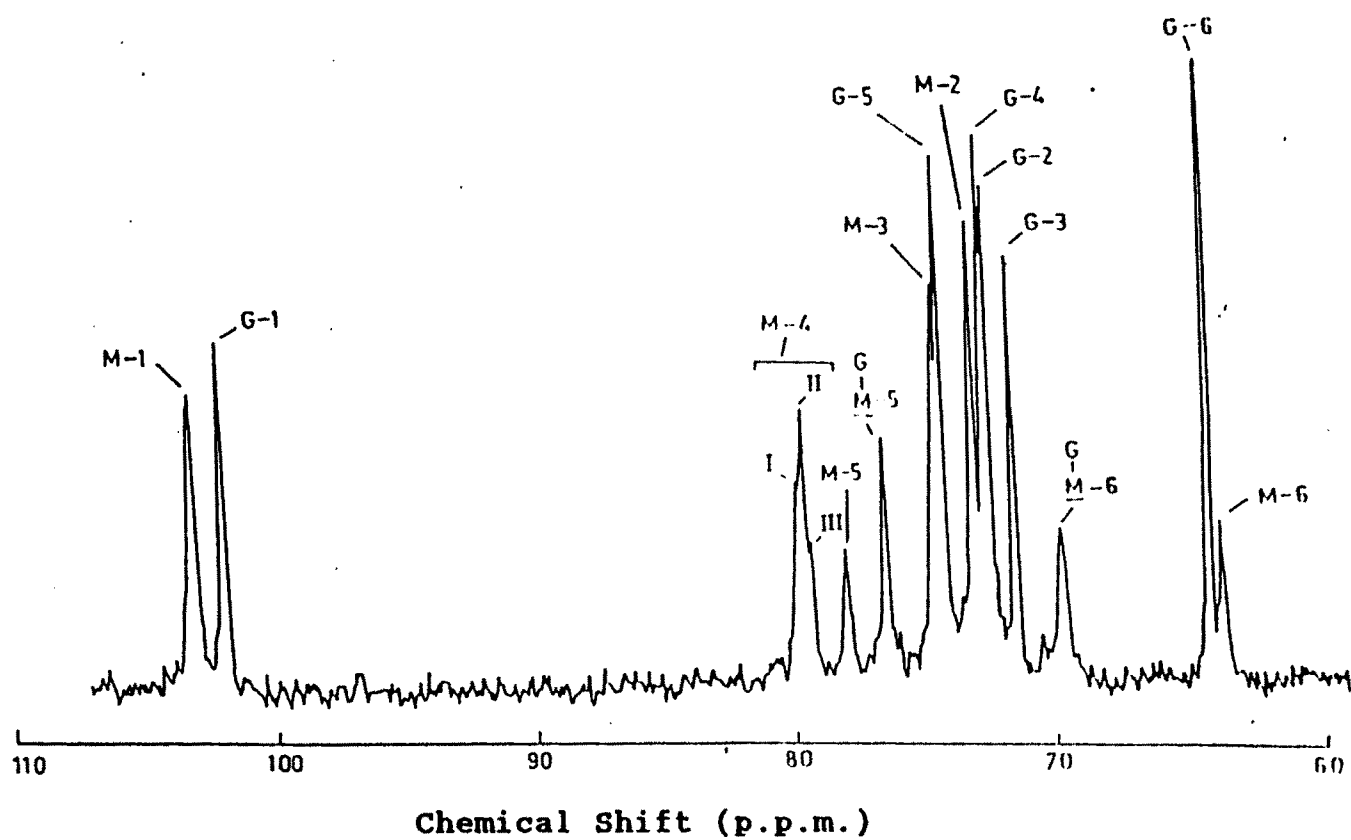
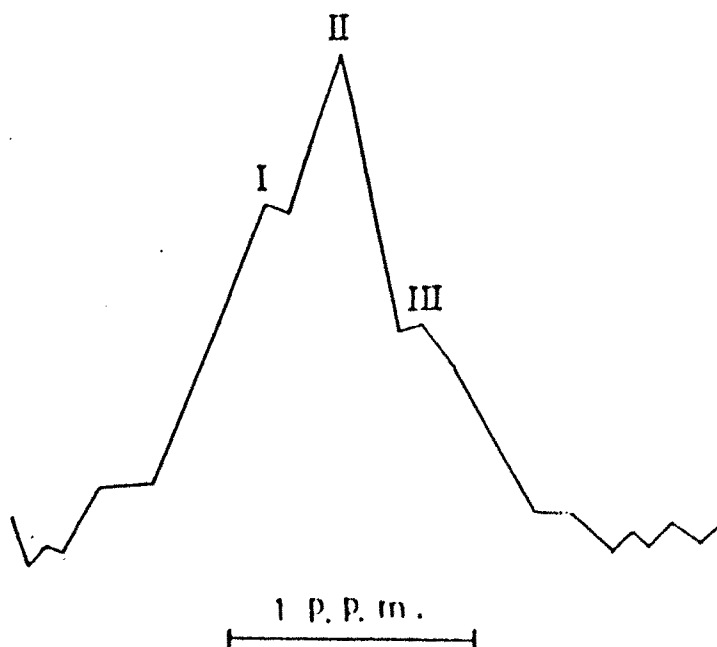


Fig.6.2 :  $^{13}\text{C}$  NMR Spectrum (25 MHz) of a solution (25mg/0.4ml) of *S. grandiflora* seed galactomannan in  $\text{D}_2\text{O}$  at  $95^\circ\text{C}$ .



**Fig.6.3 : C-4 (Man) Spectral Region**

[Interpretation (the unit involved is underlined) :

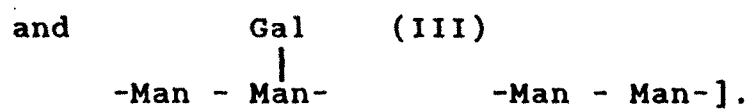
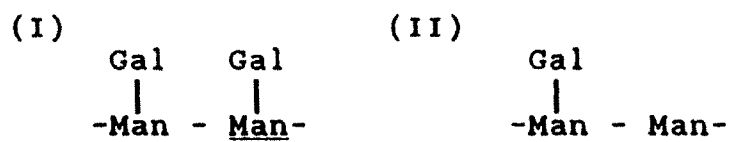


TABLE 6.1

Assignments of peaks in  $^{13}\text{C}$  NMR spectrum of *S.grandiflora* seed galactomannan

Type of Unit	Chemical shift <sup>a</sup>					
	C-1	C-2	C-3	C-4	C-5	C-6
$\alpha$ -D-Galactosyl	101.88	72.37 <sup>b</sup>	71.49	72.48 <sup>b</sup>	74.14	64.19
4-O-Substituted $\beta$ -D-mannosyl	103.01	72.92	74.47	79.34 <sup>c</sup>	78.01	63.65
4,6-Di-O- substituted $\beta$ -D-mannosyl	103.01	72.92	74.47	79.67 <sup>c</sup>	76.35	69.61
				79.67 <sup>d</sup>		

<sup>a</sup> In p.p.m.; <sup>b</sup> These are assignments may have to be interchanged; <sup>c</sup> When the preceding D-mannosyl unit is unbranched; <sup>d</sup> when the preceding D-mannosyl unit is branched.



**TABLE 6.2**  
**Compositions and diad frequencies for *S. grandiflora* seed galactomannan**

Gal/Man.Ratio	Diad Frequencies		
	F <sub>1,1</sub>	F <sub>1,2</sub> = F <sub>2,1</sub>	F <sub>2,2</sub>
0.56 <sup>a</sup>			
0.58 <sup>b</sup>	0.336	0.243	0.176
0.56 <sup>c</sup>			
0.579 <sup>d</sup>	(0.335)	(0.243)	(0.177)

(a) <sup>1</sup>H NMR; (b) <sup>13</sup>C NMR; (c) periodate oxidation; (d) calculated for a random distribution from the Gal:Man ratio obtained by <sup>13</sup>C NMR spectroscopy.