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STRUCTURE OF GALACTOMANNANS FROM *Gleditsia delavayi* AND *G. aquatica* BY ^{1}H AND ^{13}C NMR SPECTROSCOPY

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The structure of galactomannans isolated from seeds of G. delavayi *and* G. aquatica *was studied by ^{1}H and ^{13}C NMR spectroscopy. It was found that the galactomannans consisted mainly of* β*-1-4-bound mannopyranoses, a part of which was substituted on the C-6 hydroxyl by terminal units of* α*-galactopyranose.*

Key words: galactomannan, *Gleditsia delavayi*, *G. aquatica*, ^{1}H and ^{13}C NMR spectroscopy.

We have previously studied the structure of *Gleditsia* galactomannans (GM) [1-4]. We continued this research on GM isolated from seeds of *G. delavayi* (GMD) and *G. aquatica* (GMA). Our goal was to obtain data on the structure of GM from these two *Gleditsia* species using NMR spectroscopy.

GMD and GMA form viscous solutions because of their high molecular weights. This degrades significantly the resolution of the ^{13}C NMR spectrum. Therefore, we studied spectra of partially hydrolyzed GMD and GMA. In this instance, the ratio of monosaccharides does not differ sharply from the initial values (Table 1).

GM were investigated using one-dimensional ^{1}H and ^{13}C NMR spectroscopy and two-dimensional (2D) homonuclear ^{1}H/^{1}H COSY, TOCSY, ROESY, and heteronuclear ^{1}H/^{13}C HSQC and HSQC-TOCSY. The ^{13}C NMR spectrum of GMD and GMA polysaccharides contained resonances for three anomeric C atoms (δ_C 100.6, 101.4, and 100.3).

Two signals at δ_H 4.78 and 5.06 were observed in the region for resonance of anomeric protons in the ^{1}H NMR spectrum. The more resolved ^{1}H NMR spectrum of GMD polysaccharide was solved using 2D COSY and TOCSY (Table 2).

Analysis of the positions and fine structure of correlation peaks in the 2D spectra showed that the polymer consists of manno- and galactopyranose units. The resonances for H-1 of mannopyranose units (δ_H 4.78) are consistent with their β-configuration; the chemical shift of the galactopyranose H-1 units (δ_H 5.06) - the α-configuration. The 2D ROESY spectrum of the polymer contained intra-unit correlation peaks and *trans*-glycoside peaks δ_H/δ_H 4.78/3.87 (H-1 Man/H-4 Man) and 5.06/3.955 (H-1 Gal/H-6 Man). This proves the presence of a 1→4-bond between units of mannopyranose and a 1→6-bond between units of galactopyranose and mannopyranose. Signals in the ^{13}C NMR spectrum (Table 3) were assigned by analyzing the HSQC spectrum and were confirmed by substituting all mannopyranose units on C-4 and partially on C-6. The magnitude of the chemical shifts for C-5 of mannopyranose units substituted only on C-4 (δ_C 76.5) confirmed independently their β-configuration. The strong-field chemical shift of C-1 (δ_C 100.3) was consistent with the α-configuration of galactopyranose units.

The remaining chemical shifts of galactopyranose units have the same magnitude as those for the α-methyl-galactopyranoside. This is consistent with the absence of any substitution in them.

Thus, GMA polysaccharide is a galactomannan with *b*-1→4-bonds typical for plant polysacharides between mannopyranose units where part of them is substituted at the C-6 hydroxyl by terminal *a*-galactopyranose units. GMA polysaccharide gives a poorly resolved ^{1}H NMR spectrum. This hindered its investigation by 2D spectroscopic methods. However, signals in the ^{13}C NMR spectrum of the examined polysaccharides were completely identical and identified GMA as a polysaccharide of the same structure as GMD. The difference in the structure of the polysaccharides is obviously due largely

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TABLE 1. Properties of Initial and Depolymerized Galactomannans from *Gleditsia* Seeds

Main parameters	*G. aquatica*		*G. delavayi*	
	Initial	Depolymerized	Initial	Depolymerized
Gal:Man ratio	1:2.6	1:2.4	1:1.7	1:1.7
MW	-	58000	-	78000
η_{char} (*c* 0.5%, H_2O)	7.52	1.88	9.68	2.42

TABLE 2. ^{1}H NMR Chemical Shifts of *G. delavayi* Galactomannan Polysaccharides

Unit	^{1}H chemical shifts, δ, ppm						
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
→4)-β-D-Man*p*-(1→	4.78	4.16	3.82	3.87	3.59	3.955	3.80
→4)-β-D-Man*p*-(1→ 6) ↑	4.78	4.16	3.85	3.87	3.80	4.01	3.83
α-D-Gal*p*-(1	5.06	3.87	3.99	4.05	3.93	3.79	3.79

TABLE 3. ^{13}C NMR Chemical Shifts of *G. aquatica* Galactomannans

Unit	^{13}C chemical shifts, δ, ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
→4)-β-D-Man*p*-(1→	101.6; 101.4*	71.5; 71.4*	72.9	77.9	76.5	62.0
→4)-β-D-Man*p*-(1→ 6) ↑	101.6	71.5	72.8	78.3	74.8	68.0
α-D-Gal*p*-(1	100.3	69.9	70.9	70.75	72.7	62.6

*For the unit glycosylating the 4,6-substituted mannopyranose.

to polymerization of GMA. This was evident in an additional broadening of the spectral lines in both the ^{1}H and ^{13}C NMR spectra of the latter compared with those in the spectra of GMD.

Our results and those in the literature for *Gleditsia* GM [2-8] lead to the conclusion that GMD and GMA GM consist of β-1→4-bound mannopyranose units that are substituted in the 6-position by galactopyranose units with an α-1→6-bond and differ from GM known in the literature by the degree of polymerization, molecular weight, and degree of substitution of the main chain by D-galactose units.

EXPERIMENTAL

NMR spectra were recorded on Bruker AM-300 and DRX-500 spectrometers as solutions in D_2O (99.96%) at 50°C. Chemical shifts were calculated relative to acetone as an internal standard (δ_H 2.225 and δ_C 31.45). 2D spectra were recorded on a Bruker DRX-500 instrument using standard Bruker methods. The relaxation time in ROESY spectra was 30 ms.

Ultracentrifugation was performd in a MOM-3170 instrument (50,000 rpm) at 20°C for 30 min. We studied aqueous solutions (0.5%) of GM.

Partial Hydrolysis of GMD and GMA. GMD and GMA (0.1 g each) were dissolved in water (10 mL), treated with HCl (2.8 mL, 1 N), and hydrolyzed for 30 min at 85°C. The hydrolysates were centrifuged. The centrifugates were precipitated with alcohol (1:2). The resulting solids were separated, washed until neutral with alcohol, and dried. Yield of GMD and GMA, 0.06 and 0.054 g, respectively.

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