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GALACTOMANNANS FROM TWO SPECIES OF *Gleditsia* STUDIED BY ^{13}C NMR

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Galactomannans isolated from the seeds of Gleditsia macracantha *(GMM) and* Gleditsia texana *(GMT) have molecular masses (MM) of 750,000 and 795,000 and galactose to mannose ratios of 1.0:4.9 and 1.0:3.8, respectively. GMT and GMM are depolymerized to fragments of MM 25 and 19 kDa with retention of the primary structure and are studied by ^{13}C NMR spectroscopy. The principal chains of GMM and GMT are β-1-4-mannopyranose residues in which the hydroxyl groups of C-6 are substituted by single α-D-galactopyranose units.*

We previously reported a study of carbohydrates and proteins isolated from seeds of the large-thorned locust (*Gleditsia macracantha*) [1]. The goal of the present work was to isolate galactomannans from *G. macracantha* and *G. texana* and to establish their primary structure using ^{13}C NMR spectroscopy.

Aqueous extracts of the ground husk from seeds of both species were precipitated with ethanol. Water-soluble polysaccharides (WSPS) were obtained in yields of 21 and 23.7%, respectively. Hydrolysates of the WSPS were examined by GLC. Galactose and mannose were found in the ratios 1.0:4.9 (*G. macracantha*) and 1.0:3.8 (*G. texana*). Therefore, the WSPS are galactomannoses and are designated as GMM (*G. macracantha*) and GMT (*G. texana*).

The IR spectra of GMM and GMT contain absorption bands similar to those in the spectra of known 1,4-β-mannose-containing polysaccharides [2, 3]. Absorption bands are observed at 900, 870, 820, and 720 cm^{-1} in the low-frequency region. Of these, the first three indicate the presence of mannose, the β-configuration of the anomeric centers, and the pyranose ring, respectively [3]. The band at 720 cm^{-1} together with the positive specific rotation of GMM and GMT $[\alpha]_D^{20}$ +14° (*c* 0.1, H_2O) indicate that the second carbohydrate component, *D*-galactose, has the α-configuration at the anomeric center.

The galactomannans form solutions of high viscosity (η_{char} 7.5 and 9.8) owing to their high molecular masses. This prevents the recording of high-resolution ^{13}C NMR spectra. Therefore, the galactomannans were partially depolymerized by 0.1 N HCl for 45 min at 85°C. The IR spectra of the depolymerized galactomannans were identical to those of the starting GMM and GMT. The ratios of the monomers were also unchanged: 1.0:4.9 for GMM and 1.0:3.97 for GMT. However, the molecular masses were greatly decreased, to 25,000 (GMT) and 19,000 (GMM) (Table 1).

Resonances in the ^{13}C NMR spectrum of the galactomannans occur in the range 60.0-105.0 ppm (Fig. 1). The signals at 61.76 and 100.0 ppm correspond to C-6 and C-1 of the hexoses. The position of the signal for C-6 of the galactose at 62.39 (GMM) and 62.49 ppm (GMT) is consistent with the pyranose isomer and also indicates that the CH_2OH group of the galactopyranose is unsubstituted. The chemical shifts of C-2, C-3, C-4, and C-6 suggest that the hydroxyl groups of these C atoms are unsubstituted [4] (Table 2). The data presented support the conclusion that the galactopyranoses in the polysaccharide are branched through the CH_2OH hydroxyl group of the mannose.

The mannose units give many more signals in the spectrum. At strong field we observe two signals, of which the one at 61.76 ppm belongs to the C atom of the unsubstituted CH_2OH group. The signal at weaker field (67.75 for GMT and 67.76 for GMM) indicates that the mannnose units are substituted at C-6 ($\Delta\delta$ = +5.66 ppm). The positions of these signals are consistent with the pyranose isomer of the mannose. The chemical shift for C-6 of the substituted mannopyranose units indicates that the α-anomer of galactopyranose attaches at this position.

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TABLE 1. Comparative Properties of Starting and Depolymerized Galactomannans

Parameter	Galactomannans			
	starting		depolymerized	
	GMM	GMT	GMM	GMT
Gal:Man ratio	1.0:4.9	1.0:3.8	1.0:4.97	1.0:3.97
$[\alpha]_D$, deg (*c* 0.1, H_2O)	+ 14°	+ 14°	+ 24°	+ 27°
MM	750000	795000	19000	25000
η_{char}	7.50	9.87	1.93	2.50

TABLE 2. Assignment of ^{13}C NMR Signals of Galactomannans

Galactomannan	Monosaccharide unit	C-1	C-2	C-3	C-4	C-5	C-6
GMM	α-*D*-Galp	100.07	69.64	70.60	71.15	72.45	62.39
GMT		100.0	69.67	70.56	70.57	72.54	62.49
GMM	β-*D*-Manp, unsub.	101.45	71.15	71.15	77.68	76.26	61.76
GMT		101.42	71.18	72.69	78.02	76.30	61.77
GMM	β-*D*-Manp, sub.	101.28	71.76	72.65	77.98 78.18	74.59	67.76
GMT		101.51	71.17	72.69	78.31 78.19	74.47	67.75

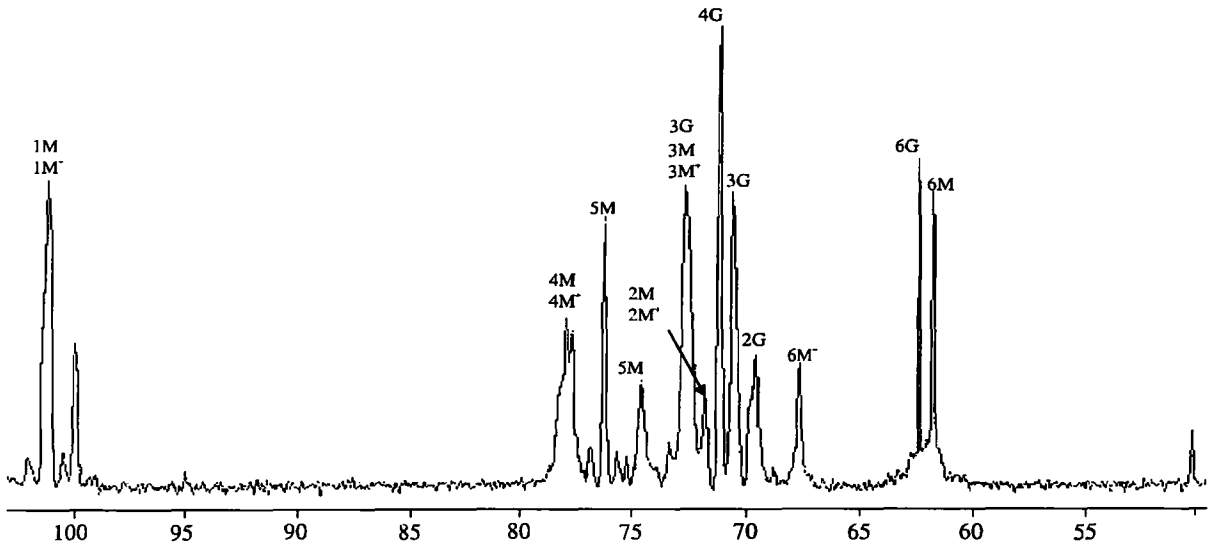

Fig. 1. ^{13}C NMR spectrum (100 MHz) of depolymerized galactomannan from *G. macracantha* (3% solution in D_2O): 4-O-substituted mannose (M), 4,6-di-O-substituted mannose (M^+), galactose (G). Numbers denote the numbers of the monosaccharide C atoms.

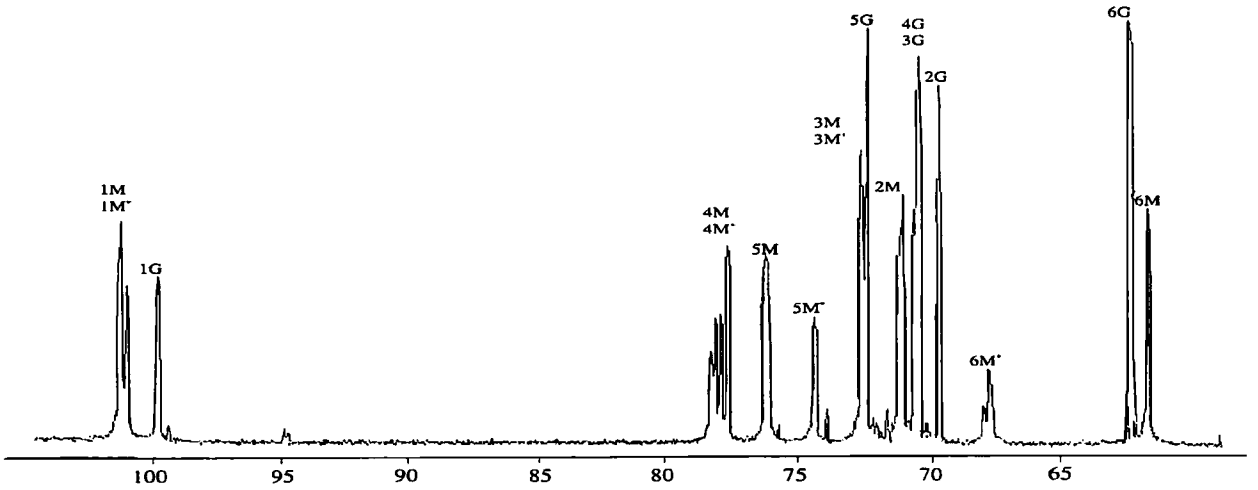

Fig. 2. ^{13}C NMR spectrum (100 MHz) of depolymerized galactomannan of *G. texana* (3% solution in D_2O): 4-O-substituted mannose (M), 4,6-di-O-substituted mannose (M^+), galactose (G). Numbers denote the numbers of the monosaccharide C atoms.

Important information is derived from the signal of C-4, which appears as three separate resonance lines (Figs. 1 and 2) and is shifted to weak field by 9.88 ppm compared with the same signal in free mannopyranose [5]. The shift to weak field indicates that the mannopyranose units are substituted at C-4, i.e., the mannose units are 1-4-bonded. The resonance lines for C-4 at strong field (77.68 ppm for GMM and 78.02 ppm for GMT) belong to unsubstituted Man-Man. The signals at 77.98 and 78.19 ppm are from Man-Man-Gal. The signals at 78.17 and 78.31 ppm are characteristic of the doubly substituted grouping Man-Man-$(Gal)_2$. This is confirmed by examining the signal for C-5 at 76.26 ppm for unsubstituted and 74.59 ppm for substituted mannopyranoses, the second of which is shifted to strong field by $\Delta\delta = -2.61$ ppm.

The data suggest that the galactomannans isolated from the seeds of *G. macracantha* and *G. texana* have main chains with β-1-4-bonded mannopyranose units substituted in the 6-position by α-galactopyranose units.

EXPERIMENTAL

General. Paper chromatography was performed on Filtrak FN-11, -7, and -1 paper using butanol—pyridine—water (6:4:3) in a descending mode. Acidic anilinium phthalate was used to locate the spots. The monosaccharides were analyzed quantitatively as the aldononitrile acetates [6]. GLC of the derivatives was carried out on a Chrom-5 apparatus with a flame-ionization detector, steel column (0.200×0.3 cm), 5% Silicone XE-60 on chromaton NAW (0.200-0.250 μm), column temperature 200°C, He carrier gas, and 60 ml/min.

The viscosity of 0.25% solutions of the galactomannans was measured using an Ostwald viscometer with capillary diameter 0.73 mm.

IR spectra were recorded on an Perkin—Elmer 2000 IR-Fourier spectrometer as pressed KBr pellets using 100 scans. The specific rotation was determined on a Zeiss polarimeter in a 1 dm tube of 10 ml volume.

^{13}C NMR spectra were recorded on a Varian UNITY 400 Plus spectrometer with a working frequency of 100 MHz for C using 3% solutions of the polysaccharide in D_2O. Methanol was used as an internal standard.

Isolation of the galactomannans was performed according to the literature [1, 3].

Hydrolysis of the galactomannans was effected using 2 N H_2SO_4 for 8 h at 100°C.

Depolymerization of the Galactomannans. Galactomannan (0.5 g) was dissolved in HCl (0.1 N, 50 ml) and hydrolyzed at 85°C for 45 min. The hydrolysate was cooled to room temperature and precipitated by ethanol in a 1:3 ratio. The precipitate was removed by centrifugation, washed with ethanol, and dried in vacuum over P_2O_5. Yields of the depolymerized galactomannans of GMM and GMT are 56 and 65.4% (of starting WSPS), respectively.

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