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ARABINOGALACTOMANNAN FROM *Gleditsia macracantha* SEEDS

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A polysaccharide with MW 25,000 consisting of arabinose, galactose, and mannose units in a 1:2.8:3.2 ratio was isolated from Gleditsia macracantha *seeds. Chemical and spectral methods established that the polysaccharide was a branched galactomannan with side branches consisting of arabinose units.*

Key words: *Gleditsia macracantha*, mannose, galactose, arabinose, arabinogalactomannan, ^{13}C NMR and PMR spectroscopy.

We previously reported the isolation and study of a water-soluble polysaccharide (WSPS) from *Gleditsia macracantha* seeds [1-3]. In continuation of these studies, total polysaccharides were obtained after isolation and precipitation of the WSPS by alcohol from the aqueous alcohol fraction. Paper chromatography (PC) of the hydrolysate of the former detected galactose, glucose, mannose, xylose, and arabinose. Hypothesizing that this polysaccharide could be a mechanical mixture of a galactomannan with other polysaccharides, an aqueous solution of the latter was fractionated using Fehling solution. The resulting precipitate (galactomannan) was separated. The supernatant solution was neutralized, evaporated to a certain volume, and dialyzed. The undialyzed part was condensed to a syrup and precipitated with alcohol to afford a water-soluble amorphous powder. The polysaccharide had molecular weight 25,000 according to sedimentation analysis. PC and GC of the hydrolysate identified mainly arabinose, mannose, and galactose in a 1:3.2:2.8 ratio. Therefore, the polysaccharide was an arabinogalactomannan (AGM). The isolation of an arabinan from *G. macracantha* seed endosperm has been reported [4].

The IR spectrum of the AGM contained absorption bands at 880 cm^{-1} (β-glycoside bond), 815 (pyranose ring), and 720 (α-glycoside bond).

The structure of the AGM was studied by periodate and chromic oxidation and methylation. Data from oxidation of the acetylated AGM by chromic anhydride [5] showed that galactose and arabinose in the polysaccharide had the α-configuration of the glycoside bond.

Oxidation of the polysaccharide by sodium periodate and subsequent Smith degradation detected erythritol, arabinose, and an insignficiant quantity of galactose. The presence of erythritol was consistent with a 1-4-bond between monosaccharide units; the presence of monosaccharides, of possible branching in the chain.

Methylation of the polysaccharide was performed using the Hakomori method [6] and produced the fully methylated product with $[\alpha]_D$ −15.8° (*c* 0.9%, acetone) and OCH_3, 40.8%. TLC of the hydrolysate of the permethylate detected 2,3,4,6-tetra-*O*-Me-D-galactose; 2,3,4,6-tetra-*O*-Me-D-mannose; 2,3,6-tri-*O*-Me-D-mannose (the main product), and insignificant quantities of 2,3,4-tri-*O*-Me-D-galactose and 2,3,5-tri-*O*-Me-L-arabinose.

The structure of the AGM was investigated by PMR and ^{13}C NMR spectroscopy. Resonances in one-dimensional spectra were assigned using two-dimensional homonuclear methods ^{1}H—^{1}H COSY, TOCSY, and ROESY in addition to heteronuclear ^{1}H—^{13}C HSQC and HSQC-TOCSY.

PMR spectra in the range of protons on anomeric C atoms exhibited four resonances at δ_H 4.78 (strongest), 5.06, 5.02, and 5.13 (the last two were minor). The other proton resonances were located in the range 3.4-4.2 ppm.

The ^{13}C NMR spectrum in the range of anomeric C atoms also showed four resonances with δ_C 101.7 (strongest), 101.5, 100.4, and 109.1 (minor).

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TABLE 1. Chemical Shifts in the PMR Spectrum of Arabinogalactomannan from *G. macracantha*

Unit	δ_H, TSP 0.0					
	H-1 H-1′	H-2	H-3	H-4	H-5	H-6 H-6′
→4)-*β*-Man*p*-(1→	4.78	4.17	3.85	3.87	3.59	3.96
→4)-*β*-Man*p*-(1→ 6) ↑	4.78	4.17	3.85	3.87	3.80	4.00, 3.85
α-Gal*p*-(1	5.06, 5.02[a]	3.87	3.99	4.04	3.94	3.81, 3.81
α-Ara*f*-(1→6)	5.13					

[a]Value for unit substituted by *α*-arabinofuranose.

TABLE 2. Chemical Shifts of C Atoms in the ^{13}C NMR Spectrum of Arabinogalactomannan from *G. macracantha*

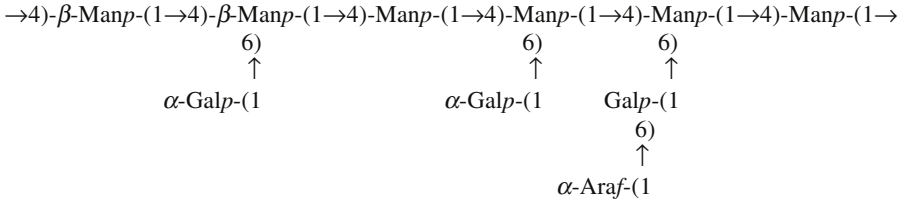
Unit	δ_C, acetone 31.45					
	C-1 C-1′	C-2	C-3	C-4	C-5	C-6
→4)-*β*-Man*p*-(1→	101.7, 101.5[b]	71.3	72.9	78.0	76.6	62.0
→4)-*β*-Man*p*-(1→ 6) ↑	101.7, 101.5[b]	71.3	72.9	78.3	74.8	68.0
α-Gal*p*-(1	100.4	69.9	70.9	70.8	72.7	62.6
α-Ara*f*-(1→6)	109.0					

[b]Value for unit glycosylating 4,6-substituted mannopyranose.

Solution of the two-dimensional spectra enabled the main resonances in the one-dimensional spectra to be assigned (Tables 1 and 2). The combined data on ^1H and ^{13}C chemical shifts indicated that the polysaccharide contained 4-substituted *β*-mannopyranose units (the main component of the chain), 4,6-disubstituted mannopyranose, terminal *α*-galactopyranose units, and *α*-arabinofuranose units as a minor component. Analysis of the ROESY spectra showed that the main polymer chain was formed from 1,4-bonded mannopyranose units, a part (about 30%) of which was substituted at the C-6 hydroxyl by *α*-galactopyranose units. The *α*-arabinofuranose units could not be reliably located in the polymer chain because of their low (<5%) content.

Splitting of the H-1 resonance of *α*-galactopyranose in the PMR spectrum into main (5.06 ppm) and minor (5.02 ppm) resonances suggested that the *α*-arabinofuranose units were bonded to *α*-galactopyranose units through the C-6 hydroxyl, as was observed previously for other plant arabinogalactomannans.

Thus, the isolated *G. macracantha* polysaccharide arabinogalactomannan was a branched galactomannan with side branches consisting of arabinofuranose units bonded to galactose through C-6 and possibly with the structure **1**.



EXPERIMENTAL

TLC was performed on Silufol UV-254 plates using solvent systems (by vol) $CHCl_3$:CH_3OH (9:1, 1) and benzene:acetone (2:1, 2). PC used Filtrak FN-12 paper and the solvent system BuOH:Py:H_2O (6:4:3, 3). Compounds were detected by spraying with anilinium biphthalate (1), KIO_4:$KMnO_4$:benzidine (2), and conc. H_2SO_4 (3).

GC was carried out in a Chrom-5 chromatograph with a flame-ionization detector, stainless-steel column (200 × 0.3 cm), XE-60 Silicone (5%) on Chromaton NAW (0.200-0.250 mesh), 210°C, and He carrier gas at 60 mL/min. Aldononitrile acetates were prepared as before [7].

^{13}C NMR spectra in D_2O (99.96%) were recorded at 50°C on Bruker AM-300 and DRX-500 spectrometers. Chemical shifts were calculated from the acetone resonance as an internal standard (δ_H 2.225, δ_C 31.45). Two-dimensional resonances were measured on a Bruker DRX-500 instrument using standard methods of the company. The relaxation time in ROESY spectra was 30 ms.

Ultracentrifugation was carried out in a MOM-3170 instrument (50,000 rpm) at 200°C for 30 min using an aqueous solution of arabinogalactomannan (1%).

Chromic Anhydride Oxidation. AGM (0.1 g) was dissolved in formamide (4 mL), treated with anhydrous pyridine (4 mL) and dropwise with acetic anhydride (4 mL), and stirred for 5 d. The acetate was precipitated with icewater (distilled). The precipitate was separated and washed with methanol and acetone. Yield 0.12 g.

The product (0.1 g) was treated with CrO_3 (0.2 g) and glacial acetic acid (3 mL), heated for 3 h at 50°C, diluted with water, and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The solid was dissolved in H_2SO_4 (1 M, 3 mL) and hydrolyzed for 6 h on a boiling-water bath. The hydrolysate was worked up as usual. PC (system 3, detector 1) identified arabinose and galactose.

Periodate Oxidation of AGM. AGM (0.02215 g) was dissolved in water (16.6 mL) and treated with $NaIO_4$ solution (3.4 mL, 0.25 M). The oxidation was carried out at +4°C for 21 d. PC (system 3, detectors 1 and 2) of the reaction prodcuts after reduction with $NaBH_4$ and hydrolysis detected erythritol, arabinose, and traces of galactose.

Methylation. AGM (0.03 g) was methylated by the Hakomori method [4] to afford the permethylate (0.021 g). The completeness of methylation was checked using TLC (systems 1 and 2, detector 3) and IR spectra (absence of absorption bands).

The permethylate (0.01 g) was refluxed in formic acid (1 mL, 85%) for 1 d, cooled, and evaporated. The solid was dissolved in H_2SO_4 (2 mL, 0.5 N) and hydrolyzed for 16 h at 100°C. The hydrolysate was worked up as usual. The products were studied by TLC (systems 1 and 2, detector 1).

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