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POLYSACCHARIDES FROM Fabaceae. IV. GALACTOMANNAN FROM *Gueldenstaedtia monophylla* SEEDS

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Galactomannan (yield 1.73% of seed mass) with molecular weight 808 kDa was isolated from seeds of Gueldenstaedtia monophylla *Fisch. (Fabaceae). Its solutions were typically highly viscous with [η] 664.7 mL/g and optically active with $[\alpha]_D$ +45.2°. It consisted of galactose and mannose in a 1:1.91 ratio. Physical chemical methods established that the principal chain of the polysaccharide consisted of 1,4-β-D-mannopyranose units substituted 50.1% in the C-6-position by single α-D-galactopyranose units and traces of 6-O-α-galactopyranosylgalactopyranose. The contents of mannobiose blocks substituted by galactose Man–Man, (Gal)Man–Man/Man–Man(Gal), and (Gal)Man–Man(Gal) in the galactomannan macromolecule were 23, 42, and 35%, respectively.*

Keywords: *Gueldenstaedtia monophylla*, Fabaceae, galactomannan, ^{13}C NMR spectroscopy.

Gueldenstaedtia Fisch. (Fabaceae) is a genus of the Astragalinae subtribe and is closely related to the genera *Astragalus*, *Oxytropis*, *Biserrula*, and *Alhagi* [1]. According to the latest taxonomic studies, this genus includes four species and one subspecies: *G. monophylla*, *G. taihangensis*, *G. henryi*, *G. verna*, and *G. verna* f. *alba* [2]. *G. monophylla* is a rare, relict, ecologically narrowly specialized, and endemic species that is encountered in small populations of Altai and Tuva that are diminishing [3]. The chemistry of this species has not been previously studied. Our preliminary experiments found galactomannans in *G. monophylla* seeds. Herein we report the isolation and structural characterization of the galactomannan from *G. monophylla* seeds.

Galactomannan from *G. monophylla* seeds (GMGm) was isolated in 1.73% (of seed mass) yield from defatted raw material by aqueous extraction followed by precipitation by Fehling solution, demineralization, and deproteination. The resulting polysaccharide was very soluble in water and produced highly viscous ([η] 664.7 mL/g) and optically active ($[\alpha]_D$ +45.2°) solutions. Total hydrolysis showed that GMGm consisted of galactose and mannose in a 1:1.91 ratio. The molecular weight of GMGm as determined by viscosimetry was 808 kDa. The IR spectrum contained bands characteristic for galactomannans [4].

The polysaccharide was acetylated and treated subsequently with CrO_3 in order to determine the configuration of the galactose and mannose. The hydrolysate of the oxidation product contained only galactose, which was possible for the α-configuration. The lack of mannose was consistent with its β-configuration.

The hydrolysate of methylated GMGm contained 2,3,4,6-tetra-*O*-Me-Gal*p*, 2,3-di-*O*-Me-Man*p*, 2,3,6-tri-*O*-Me-Man*p*, and 2,3,4-tri-*O*-Me-Gal*p* in a 19.03:19.23:19.12:1 ratio. These results indicated that the main chain of GMGm included (1→4)-bound mannopyranose units substituted 50.1% in the C-6-position by single galactopyranose units. The tri-substituted galactopyranose was observed because the side chains of the macromolecule included not only single galactose units but also longer chains of them.

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TABLE 1. ^{13}C NMR Spectra of Depolymerized Galactomannan GMGm-d

GMGm-d	^{13}C chemical shifts, ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
α-Gal*p*-1→	99.50	69.17	70.18	70.34	72.07	62.40
→6-α-Gal*p*-1→	99.14	69.42	70.18	70.34	72.07	68.14
→4-β-Man*p*-1→	101.91	71.41	72.16	76.15 77.06	74.80	61.81
→4,6-β-Man*p*-1→	100.76	71.41	72.16	77.06 77.75	73.17	67.54

Partial acid hydrolysis of the polysaccharide with subsequent isolation of the resulting products was carried out in order to determine the nature of the substitution in the galactose chains in GMGm side chains. Chromatographic separation identified galactose, mannose, 4-*O*-β-D-mannopyranosyl-D-mannopyranose, 4-*O*-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-D-mannopyranose, 6-*O*-α-D-galactopyranosyl-D-mannopyranose, and traces of 6-*O*-α-D-galactopyranosyl-D-galactopyranose and 6-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-D-mannopyranose. The similar type of splitting indicated that GMGm contained as side substituents not only single galactopyranosyl units but also a certain amount of galactobiosyl chains in which the galactose units were α-(1→6)-bound.

The GMGm was studied further using ^{13}C NMR spectroscopy of depolymerized GMGm-d with similar physical chemical properties but smaller molecular weights (Table 1). The galactose resonances were consistent with the α-configuration because the C-1 resonance was shifted to weak field (99.50 ppm) and the pyranose ring was present (C-6 resonance at 62.40 ppm). The chemical shifts of C-2–C-6 were similar to those of free α-D-galactopyranose [5]. A minor resonance at 68.14 ppm was assigned to C-6 from a trace of 1,6-bound galactopyranose of galactobiose side chains. The ratio of integrated intensities of the C-6 resonances from single and galactobiose units was 19:1, which was close to the results obtained by methylation.

The mannose units in GMGm had the β-configuration. This was confirmed by the chemical shift of C-5 at 74.80 ppm [6]. The resonances of C-1, C-4, and C-6 were shifted to weak field relative to free β-D-mannopyranose by 7.01, 8.25, and 4.85 ppm, respectively, indicating that they were involved in bond formation. The chemical shift of C-6 in substituted mannose (67.54 ppm) indicated that the substituent had the α-configuration [7]. Moreover, mannose had the pyranose form because the C-6 resonance of unsubstituted units was found at 61.81 ppm. Resonances of mannopyranose C-4 provided information about the fine structure of the GMGm polymer chain [8]. The ratio of integrated intensities of resonances at 76.15, 77.06, and 77.75 ppm, which were due to mannose blocks substituted by galactose, was 1:1.83:1.52. Thus, the contents of the mannobiose block unsubstituted by galactose Man–Man and the total of the two singly substituted blocks (Gal)Man–Man and Man–Man(Gal) and the doubly substituted block (Gal)Man–Man(Gal) were 23, 42, and 35%, respectively.

The results as a whole enabled the structure of the galactomannan from *G. monophylla* seeds to be determined as a polymer containing in the main chain α-(1→4)-mannopyranose units substituted 50.1% by single β-galactopyranose units (~90%) and 6-*O*-α-galactopyranosylgalactopyranose (~10%). Galactomannans of similar structure were isolated previously from seeds of *Cassia angustifolia* [9], *Gleditsia ferox* [10], *G. amorphoides* [11], and *Trifolium repens* [12].

EXPERIMENTAL

Seeds of *G. monophylla* were collected in July 2007 in Ongudai Region (Banks of the Inegen River, Altai Territory, July 15, 2007, 820 m above sea level, 52°32′88″ N, 86°76′70″ E). The species was identified by Candidate of Biological Sciences I. Yu. Selyutina (CSBG, SD, RAS). Seeds were stored in the seed bank at the Division of Biologically Active Compounds, IGEB, SD, RAS (No. Fb/s-32/7-08/0708).

HPTLC was carried out on Sorbfil PTSKh-AF-V silica-gel plates (Imid Ltd.); PC, on FN-2 chromatographic paper using PrOH:$CHCl_3$:H_2O (1, 7:4:1, double rise to 3.5 and 7 cm) and *i*-PrOH:H_2O (2, 8:2) with detection by *p*-hydroxydiphenyl phosphate (1%). Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optical-Mechanical Plant) in a 1-dm

cuvette at 20°C. IR spectra were recorded in films on TlBr-I glass plates (KRS-5) in the range 4000–450 cm^{-1} on a Spectrum 100 IR-Fourier spectrometer (Perkin–Elmer). GC/MS of monosaccharide methyl ethers was carried out in a 5973 N GC/MS (Agilent Technologies) with a 6890N mass-selective detector (Agilent Technologies) with a difussion pump using a PH-Innowax capillary column (30 m/250 μm/0.50 μm). ^{13}C NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz. Spectra were taken in DMSO-d_6 (1% solutions). Galactose, mannose (Acros Organics), mannobiose, and mannotriose (Carbosynth Ltd.) were used as standards.

Isolation of Galactomannan from *G. monophylla*. Ground seeds of *G. monophylla* (82 g) were extracted in a Soxhlet apparatus successively by hexane, $CHCl_3$, EtOAc, and Me_2CO. The solid raw material was dried and extracted with water (3×, 1:15) on a boiling water bath for 2 h. The aqueous extracts were separated by centrifugation (6,000 g, 30 min) and combined. Water-soluble polysaccharides were precipitated by EtOH (95%, 1:4) by storing the mixture for 10 h at 10°C, centrifuging (6,000 g, 20 min), washing the precipitate with EtOH (70–95%), Me_2CO, and EtOAc, and drying. Yield 4.24 g of GMPs fraction (5.17% of seed mass). Galactomannan was isolated by precipitation from an aqueous solution of GMPs (1%) using Fehling solution with subsequent regeneration and drying [13]. Yield 1.64 g of GMGm galactomannan (2% of seed mass). The galactomannan was demineralized using KU-2-8 cation-exchanger (H^+-form, 50 × 300 mm column, water eluent) and deproteinated using treatment with pronase (*Streptomyces griseus*, KF.3.4.244, Sigma) [14]. The yield of demineralized and deproteinated GMGm galactomannan was 1.42 g (1.73% of seed mass).

Viscosimetric studies were carried out as before [15]. Molecular weights of galactomannans were calculated based on characteristic viscosity values [16].

Total Hydrolysis. GMGm (20 mg) was dissolved in TFA (2 M, 5 mL) and heated at 120°C for 2 h. The hydrolysate was concentrated in vacuo with MeOH and analyzed by HPTLC (system 1) and GC/MS (as methyl ethers).

GMGm: $[\alpha]_D$ +45.2° (*c* 1.0, H_2O), [η] 664.7 mL/g (*c* 0.8, H_2O). MW 808 kDa. Galactose:mannose ratio 1:1.91. IR spectrum (ν, cm^{-1}): 765, 813, 871, 897, 960, 1029, 1075, 1147, 1215, 1303, 1377, 2103, 2895, 3408.

Oxidation by CrO_3 of previously acetylated GMGm was carried out as before [17]. The oxidation product was hydrolyzed and analyzed by HPTLC (system 1).

Methylation of GMGm was carried out using methyliodide as before [18]; formolysis and hydrolysis of the permethylate, according to the literature method [19]. Hydrolysates were analyzed by GC/MS.

Partial hydrolysis of GMGm (850 mg) was carried out in H_2SO_4 solution (0.05 M) at 100°C for 12 h. The hydrolysate was neutralized over ARA-5pT40 anion-exchange resin (CO_3^{-2}-form), concentrated, and separated using preparative PC (system 2) to afford a series of hydrolysis products G1, G2, G3, G4, G5, G6, and G7. These were identified using chromatographic mobilities (HPTLC), melting points, total hydrolysis, and methylation.

G1, galactose; **G2**, mannose; **G3**, 4-*O*-β-D-mannopyranosyl-D-mannopyranose, mp 203°C, 2,3,6-tri-*O*-Me-Man*p*:2,3,4,6-tetra-*O*-Me-Man*p* 1:1; **G4**, 4-*O*-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-D-mannopyranose, mp 209°C, 2,3,6-tri-*O*-Me-Man*p*:2,3,4,6-tetra-*O*-Me-Man*p* 2:1; **G5**, 6-*O*-α-D-galactopyranosyl-D-mannopyranose, Gal—Man 1:1, 2,3,4,6-tetra-*O*-Me-Gal*p*:2,3,4-tri-*O*-Me-Man*p* 1:1; **G6**, 6-*O*-α-D-galactopyranosyl-D-galactopyranose, 2,3,4,6-tetra-*O*-Me-Gal*p*:2,3,4-tri-*O*-Me-Gal*p* 1:1; **G7**, 6-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-D-mannopyranose, Gal–Man 2:1, 2,3,4-tri-*O*-Me-Man*p*:2,3,4,6-tetra-*O*-Me-Gal*p*:2,3,4-tri-*O*-Me-Gal*p* 1:1:1.

Depolymerization of GMGm (78 mg) was carried out in HCl solution (1 M) by the literature method [20] to afford GMGm-d (40 mg, 51.3% of GMGm mass, Gal:Man ratio 1:1.89, MW 102 kDa, IR spectrum agreed with that of GMGm).

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