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POLYSACCHARIDES OF Fabaceae. II. GALACTOMANNAN FROM *Astragalus danicus* SEEDS

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Galactomannan of molecular weight 472 kDa was isolated from Astragalus danicus *Retz. (Fabaceae) seeds and consisted of galactose and mannose units in a 1:1.40 molar ratio. The main chain of the macromolecule was constructed of 1,4-*β*-D-mannopyranose units, 71% of which were substituted at C-6 by single* α*-D-galactopyranose units.*

Key words: *Astragalus danicus*, Fabaceae, galactomannan, ^{13}C NMR spectroscopy.

In continuation of research on polysaccharides of the family Fabaceae [1], we studied *Astragalus danicus* Retz., which is widely distributed in the Baikal region. Its chemical composition is insufficiently studied. 3-*O*-Methylinositol, linolenic acid, and several triterpene glycosides that were derivatives of 3β,22β,24-trihydroxyolean-12-ene were isolated from the aerial part [2]. Galactomannans are known to be present in *A. danicus* seeds. However, this class of reserve polysaccharides has not been studied in detail [3]. The goal of our work was to isolate and characterize the structure of galactomannan from *A. danicus* seeds.

Extraction of *A. danicus* seeds with hot water and subsequent precipitation with ethanol produced the polysaccharide complex, from which the polysaccharide ADGm was isolated in 3.39% yield (of the seed mass) after treatment with Fehling solution. ADGm was a white fibrous powder that was soluble in water to form a viscous solution. It consisted of galactose and mannose in a 1:1.40 ratio. The principal physicochemical propreties of ADGm were $[\alpha]_D$ +73.1° (*c* 0.46, H_2O), [η] 471.7 mL/g (*c* 0.5, H_2O), and molecular mass 472 kDa. The IR spectrum of the galactomannan agreed with those of previously studied biopolymers of this class [4]. It exhibited absorption bands for C–H bonds in pyranose rings and β-bonded monomers (817, 882 cm^{-1}), α-hexopyranose pyranose rings (721), and polysaccharides in general.

Periodate oxidation of ADGm consumed 1.43 mol of $NaIO_4$ per anhydro-unit and released 0.42 mol of HCOOH. Products of Smith degradation contained glycerine and erythritol in a 1:1.4 ratio. These facts were consistent with the presence of (1→4)- and (1→6)-bonds in the structure of the studied galactomannan.

Methylation of ADGm, formolysis, and hydrolysis of the resulting permethylate produced in the hydrolysate 2,3,4,6-tetra-*O*-Me-Gal*p*, 2,3,6-tri-*O*-Me-Man*p*, and 2,3-di-*O*-Me-Man*p* in a 2.49:1.00:2.50 ratio. The methylation results showed that the main chain of the galactomannan contained (1→4)-bonded mannopyranose units, about 71% of which were substituted at C-6 by single galactopyranose units.

Oxidation of ADGm acetate by chromic anhydride and subsequent hydrolysis of the oxidation products produced only galactose. This was consistent with the α-configuration of its anomeric center and the β-configuration of mannose because it was not found in the hydrolysate.

Starting galactomannan was depolymerized for further studies using ^{13}C NMR spectroscopy. The resulting product ADGm-d had similar galactose:mannose ratios, specific rotation, and IR spectra. Table 1 lists the chemical shifts of C atoms in the ^{13}C NMR spectra of the depolymerized galactomannan and their interpretation.

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TABLE 1. Chemical Shifts (ppm) of C Atoms in ^{13}C NMR Spectra of Depolymerized Galactomannan ADGm-d

	C-1	C-2	C-3	C-4	C-5	C-6
α-Gal*p*-1→	98.73	68.52	69.11	69.90	71.70	62.00
				77.63		
→4-β-Man*p*-1→	101.57	70.04	71.94	77.97	76.52	62.15
				77.97		
→4,6-β-Man*p*-1→	100.98	71.12	72.21	78.10	74.71	67.34

Analysis of the chemical shifts of the galactose C atoms revealed that the C-1 resonance was shifted by 5.23 ppm due to the α-effect of the galactoside bond in which it was involved, having the α-configuration of the anomeric center. The shifts for C-2 through C-6 differed little from those of free α-galactose. The position of the C-6 resonance (62.00 ppm) indicated that the ring was in the pyranose form, i.e., galactose was present in the galactomannan as single α-galactopyranose units [5].

Mannose had the β-configuration of the anomeric center because the chemical shift of C-5 was found near 76.52 ppm. The resonance would have been found at stronger field if the α-configuration were present [6]. The C-6 resonance of unsubstituted mannose units (62.15) was consistent with the pyranose form. Atoms C-1, C-4, and C-6 were involved in forming bonds, causing their resonances to shift to weak field (+6.08, +10.07, +5.04, respectively). The α-anomeric substituent was bonded to C-6 because its resonance was shifted to 67.34 ppm. If the substituent had the β-configuration, the shift would have been 70 ppm [7].

An examination of the mannose C-4 resonances led to a conclusion about the ratio of the various structural components in the macromolecule that was due to the presence in the polymeric chain of three types of constituents. These were the mannose constituent Man–Man unsubstituted by galactose (77.63 ppm), the sum of two singly substituted constituents (Gal)Man–Man and Man–Man(Gal) (77.97), and a doubly substituted constituent (Gal)Man–Man(Gal) (78.10) [8]. The integrated intensities of these resonances indicated that the contents in ADGm of these constituents were 18, 25, and 57%, respectively.

The results for the structure of *A. danicus* galactomannan indicated that the main chain of the polysaccharide consisted of (1→4)-bonded β-mannopyranose units partially substituted at C-6 by single α-galactopyranose units.

EXPERIMENTAL

A. danicus Retz. seeds were collected in August 2007 in Pribaikal Region of the Republic of Buryatia.

HPTLC was performed on PTSKh-AF-V Sorbfil plates (Sorbpolimer). The solvent systems were *i*-PrOH:$CHCl_3$:H_2O (7:4:1, double elution to heights of 4 and 8 cm) (1), BuOH:Py:H_2O (15:30:20) (2), and benzene:acetone (2:1) (3). Detection used *p*-hydroxydiphenylphosphate (1) and $KMnO_4$:$NaIO_4$:benzidine (2).

Optical rotation was determined on a SM-3 polarimeter (Zagorsk Optico-Mechanical Plant) in a 1-dm cuvette at 20°C. IR spectra as films on KRS-5 plates were recorded on a Spectrum 100 IR—Fourier spectrometer (Perkin—Elmer) in the range 4000-450 cm^{-1}. Spectrophotometric studies were carried out on a UV-Vis-mini spectrophotometer (Shimadzu) in 10-mm quartz cuvettes. ^{13}C NMR spectra in DMSO-d_6 solutions (1%) were recorded on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz.

Water-soluble polysaccharides were isolated from *A. danicus* seeds that were ground to a floury consistency (24 g) and defatted beforehand with hexane, $CHCl_3$, and acetone. The treated raw material was dried and extracted three times with H_2O (1:20 ratio) at 100°C for 2 h. The aqueous extracts were separated by centrifugation (5000 g, 30 min), combined, and precipitated with ethanol (1:3 ratio, 95%). After 24 h the suspension was centrifuged (5000 g, 20 min). The solid was washed with ethanol (70-95%), acetone, and ethylacetate and dried. Yield 1.76 g (7.33% of the seed mass).

Galactomannan (ADGm) was isolated using the copper complex with Fehling solution [9]. The yield of ADGm was 813 mg (3.39% of the seed mass).

Total Hydrolysis. ADGm (20 mg) was dissolved in TFA (5 mL, 2 M) and heated at 120°C for 2 h. The hydrolysate was concentrated in vacuo in the presence of MeOH and analyzed by HPTLC (system 1, detector 1). The quantitative monosaccharide composition was determined by a densitometric method [10].

Viscosimetric studies were performed as before [11]. Molecular weights of polysaccharides were calculated based on the characteristic viscosities [12].

Periodate Oxidation. ADGm (30 mg) was dissolved in water (50 mL), treated with $NaIO_4$ solution (15 mL, 0.08 M), and left at 4°C. Aliquots of the solution were taken every 24 h and analyzed for IO_4^- (spectrophotometric method [13]) and HCOOH (titration with NaOH solution, 0.01 M) contents. The reaction was stopped after 10 d by adding ethyleneglycol (2 mL). Then H_2O (20 mL) and $NaBH_4$ (200 mg) were added. The solution was treated after 12 h with cation-exchanger KU-2-8 (30 g, H^+-form). The filtrate was concentrated in the presence of MeOH to dryness. The dry solid was dissolved in H_2SO_4 solution (5 mL, 1 M) and heated at 100°C for 6 h. The hydrolysate was treated with anion-exchanger AV-17-8 (HCO_3^--form), concentrated to the minimal volume in vacuo at 40°C, and analyzed by HPTLC (system 2, developer 2).

Methylation of the studied compounds was carried out by the Ciucanu—Kerek method [14]; formolysis and hydrolysis of the permethylate, by the literature method [10]. Hydrolysates were analyzed by HPTLC (system 3, detector 1) and compared with authentic samples of methylated pyranoses.

Chromic anhydride oxidation of polysaccharides was perfromed after preliminary acetylation by the literature method [15].

ADGm Depolymerization. ADGm (200 mg) was dissolved in water (30 mL), treated with HCl (2 mL, 1 M), heated at 100°C for 2 h, and centrifuged. The supernatant was precipitated with ethanol (95%, 1:4). The resulting precipitate (ADGm-d) was washed with ethanol (80%) and dried to afford ADGm-d (109 mg, 54.5% of ADGm mass), Gal:Man, 1:1.39, $[\alpha]_D$ +75.0° (*c* 0.5, H_2O), $[\eta]$ = 69.1 mL/g (*c* 0.4, H_2O), molecular weight 75.3 kDa. The IR spectrum agreed with that of starting galactomannan ADGm.

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