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POLYSACCHARIDES OF Fabaceae. I. GALACTOMANNAN OF *Astragalus sericeocanus* SEEDS

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Galactomannan (yield 3.58% of seed mass) of molecular weight 876 kDa was isolated from seeds of Astragalus sericeocanus *Gontsch. (Fabaceae). Its solutions had high viscosity [η], 764.6 mL/g, and optical density $[\alpha]_D$ +65.3°. The polysaccharide consisted of galactose and mannose in molar ratio 1:1.58. The main chain of the galactomannan macromolecule was constructed of 1,4-β-D-mannopyranose units, 63% of which were substituted at C-6 by single α-D-galactopyranose units. ^{13}C NMR spectroscopy established that the galactomannan contained units of differently substituted galactose mannobiose units: Man-Man, (Gal)Man-Man, and/or Man-Man(Gal) in addition to (Gal)Man-Man(Gal), the ratio of which was 0.15:0.51:0.34.*

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

Seeds of the genus *Astragalus* are a valuable source of galactomannans, which have been found in 16 species of this genus [1-4]. *A. sericeocanus* Gontsch. is an endemic species of the family Fabaceae with a limited distribution in Buryatia (Russia). The chemical composition of this species has not been reported. The goal of our work was to isolate and characterize the structure of galactomannan from *A. sericeocanus*.

The total water-soluble polysaccharides (WSPS) from *A. sericeocanus* were precipitated using a copper complex to isolate the polymer ASGm in 3.58% yield of the raw material mass. Hydrolysis gave galactose and mannose in a 1:1.58 ratio, characterizing it as a galactomannan. ASGm was a white powder that was soluble in water after preliminary swelling. Its solutions were highly viscous. The main physicochemical properties of the galactomannan were $[\alpha]_D$ +65.3° (*c* 0.5, H_2O), [η] = 764.6 mL/g (*c* 0.5, H_2O), molecular weight 876 kDa. The IR spectrum of ASGm agreed with those of previously studied polysaccharides of this class [5].

Periodate oxidation of ASGm consumed 1.31 mol of periodate per single anhydro unit and released 0.33 mol HCOOH. Smith degradation of the resulting polyalcohol produced erythritol and glycerin in a 1:1.5 ratio. This was consistent with the presence of (1→4)- and (1→6)-bonds in the galactomannan structure.

Next, the galactomannan was methylated. Hydrolysis of the permethylate produced 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 1.69:1.0:1.73 ratio. The methylation data indicated that the main polymeric chain of ASGm consisted of (1→4)-bonded mannose with single galactose units in side chains that were bonded to the C-6 position of mannose. The degree of substitution of the main chain was 63%.

The configurations of the anomeric protons in the galactomannan were established using oxidation of the acetylated polymer by chromic anhydride. The hydrolysate of the oxidation product contained only galactose. This confirmed that it had the α-configuration. The absence of mannose proved that its anomeric center had the β-configuration.

The structure of ASGm was further refined by studying the ^{13}C NMR spectrum of depolymerized ASGm (ASGm-d) (Table 1).

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TABLE 1. ^{13}C NMR Spectrum of Depolymerized Galactomannan ASGm-d (δ, ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
α-Gal*p*-1→	99.42	68.84	69.33	69.70 76.78	71.69	61.82
→ 4-β-Man*p*-1→	100.73	70.18	72.03	77.22 77.22	76.80	61.09
→ 4,6-β-Man*p*-1→	100.29	70.71	72.03	77.87	75.30	66.98

The chemical shifts of the C atoms of the galactose units showed that the resonance for C-1 had shifted to weak field by 5.9 ppm (α-effect) compared with that of D-galactose. This indicated that its anomeric center had the α-configuration. The resonance for C-6 (61.82 ppm) was consistent with the pyranose form. Chemical shifts of C-2 through C-6 were practically the same as those of free α-galactopyranose. This led to the conclusion that galactose in the galactomannan was present as unsubstituted α-galactopyranose units.

The position of the resonance for C-5 of mannose (76.80 ppm) confirmed that its anomeric center had the β-configuration because the resonance would have appeared at weaker field if it had the α-configuration [6]. The position of the C-6 resonance of unsubstituted mannose units (61.09 ppm) confirmed that the ring had the pyranose configuration. Resonances of C-1, C-4, and C-6 of substituted mannose were shifted because they were involved in the formation of covalent bonds. The appearance of a second resonance for C-5 at strong field (75.30 ppm) confirmed the presence of a bond to C-6 (β-effect) [6]. The resonance of substituted C-6 at 66.98 ppm was a consequence of the bonding to an α-anomer because the presence of a β-substituent would have shifted it to greater than 70 ppm [7].

The structure of the galactomannans were refined using approaches proposed earlier by Grasdalen and Painter [8] that were based on examination of the splitting of the resonances of C-4 of the mannopyranose units. This effect is caused by the presence of three types of structural blocks in the galactomannans. These are a mannobiose block Man-Man that is unsubstituted by galactose, a total of two singly substituted blocks (Gal)Man-Man and Man-Man(Gal), and a doubly substituted block (Gal)Man-Man(Gal). The resonances of these blocks for ASGm were located at 76.78, 77.22, and 77.87 ppm, respectively. The ratio of the integrated intensities of these resonances was 0.15:0.51:0.34 (the whole chain was taken as 1). This corresponded to 15, 51, and 34% content, respectively, of these blocks in the structure of ASGm.

It should be noted in summarizing the structural data for ASGm that the main chain in the isolated polysaccharide was constructed of 1,4-bonded β-D-mannopyranose units substituted at C-6 by single α-D-galactopyranose units.

EXPERIMENTAL

Seeds of *A. sericeocanus* were collected in July 2007 in Pribaikal Region of the Republic of Buryatia. HPTLC was carried out on Sorbfil PTSKh-AF-V plates (Sorbpolimer) using solvent systems *i*-PrOH:$CHCl_3$:H_2O (double elution to heights of 4 and 8 cm) (1, 7:4:1) and benzene:acetone (2, 2:1). The developers were *p*-hydroxydiphenylphosphate (1) and $KMnO_4$:$NaIO_4$:benzidine (2).

Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optical-Mechanical Plant) in 1-dm cuvettes at 20°C. IR spectra of films on KRS-5 plates were recorded on a Spectrum 100 IR-Fourier spectrometer (Perkin—Elmer) in the range 4000-450 cm^{-1}. Spectrophotometry was performed on CE2011 (Cecil) and UV—Vis-mini (Shimadzu) spectrophotometers in 10-mm quartz cuvettes. ^{13}C NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz. Spectra in DMSO-d_6 solutions (1%) were recorded.

Isolation of WSPS was carried out using *A. sericeocanus* seeds (18 g) that were ground to a powder and defatted beforehand with C_6H_{14}, $CHCl_3$, and $(CH_3)_2CO$. The resulting raw material was dried and extracted three times with water (1:15 ratio) at 100°C for 3 h. The aqueous extracts were separated by centrifugation (5000 g, 30 min), combined, and precipitated with ethanol (95%, 1:3 ratio). The precipitate was formed by storing the mixture for 24 h at 5°C and centrifuging (5000 g, 20 min). The precipitate was washed with ethanol (70-95%), acetone, and ethylacetate and dried. Yield of WSPS, 1.53 g (8.50% of seed mass).

Isolation of galactomannan (ASGm) was carried out using a copper complex with Feling solution [9]. Yield of ASGm, 644 mg (3.58% of seed mass).

Total Hydrolysis. ASGm (20 mg) was dissolved in TFA (5 mL, 2 M) and heated at 100°C for 4 h. The hydrolysate was neutralized by anion-exchange resin AV-17-8 (HCO_3^--form), concentrated to the minimum volume in vacuo at 40°C, and analyzed by HPTLC (system 1, developer 1). The quantitative monosaccharide composition was determined by densitometry as described previously [10].

Viscosimetric studies were carried out according to the literature [11]. Molecular weights of polysaccharides were calculated based on characteristic viscosity values [12].

Periodate Oxidation. ASGm (30 mg) was dissolved in water (50 mL), treated with $NaIO_4$ solution (15 mL, 0.08 M), and stored at 4°C. An aliquot of the solution was analyzed every 24 h for IO_4^- (spectrophotometric method from the decrease of absorption at 223 nm [13]) and HCOOH (titration by 0.01 M NaOH solution) content. The reaction was stopped after 10 d by adding ethyleneglycol (2 mL), water (20 mL), and $NaBH_4$ (200 mg). After 12 h the solution was treated with KU-2-8 cation exchanger (H^+-form). The filtrate was concentrated in the presence of methanol and then taken to dryness. The solid was dissolved in H_2SO_4 (5 mL, 1 M) and heated at 100°C for 6 h. The hydrolysate was treated with AV-17-8 anion exchanger (HCO_3^--form), concentrated to the minimum volume in vacuo at 40°C, and analyzed by HPTLC (system 1, developer 2).

Methylation of the studied compounds was carried out by the Ciucanu-Kerek method [14]; formolysis and hydrolysis of the permethylate, as before [10]. Hydrolysates were analyzed by TLC (system 2, developer 1) and compared with authentic samples of methylated pyranoses.

Oxidation by chromic anhydride of polysaccharides was performed after preliminary acetylation by the literature method [15].

Depolymerization of ASGm. ASGm (250 mg) was dissolved in water (30 mL), treated with HCl (1 M) until the pH was 2.0, heated on a boiling water bath for 2 h, and centrifuged. The supernatant was precipitated with ethanol (95%, 1:4). The resulting precipitate (ASGm-d) was washed with ethanol (80%) and dried. Yield of ASGm-d, 82 mg (32.8% of ASGm mass). Gal:Man ratio, 1:1.56, $[\alpha]_D$ +66.1° (*c* 0.5, H_2O), $[\eta]$ = 58.3 mL/g (*c* 0.5, H_2O), molecular weight 63.2 kDa. The IR spectrum agreed with that of starting ASGm.

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