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Fabaceae POLYSACCHARIDES. III. GALACTOMANNAN FROM *Astragalus cicer* SEEDS

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Seeds of Astragalus cicer *L. (Fabaceae) afforded a galactomannan (5.90% yield of seed mass) of molecular weight 1064 kDa, solutions of which had high viscosity [η] 925.5 mL/g and optical activity $[\alpha]_D$ +71.9°. The galactomannan consisted of galactose and mannose units in a 1:1.39 ratio. Physicochemical methods established that the main chain of the polysaccharide consisted of 1,4-β-D-mannopyranose units substituted at 72% of the C-6 positions by single α-D-galactopyranose units. The content of variously substituted galactose mannobiose units Man–Man, (Gal)Man–Man/Man–Man(Gal) and (Gal)Man–Man(Gal) in the galactomannan were 18.7, 19.8, and 61.5%, respectively.*

Keywords: *Astragalus cicer*, Fabaceae, galactomannan, ^{13}C NMR spectroscopy.

In continuation of research on polysaccharides of plants from the family Fabaceae [1], we used *Astragalus cicer* L. [*A. mucronatum* DC., *Cystium cicer* (L.) Stev.] as raw material. The natural distribution area of this plant covers the European part of Russia and the Caucases [2]. The aerial part of *A. cicer* contains pinitol [3], astraciceran [4], kaempferol 3-*O*-rutinoside-7-*O*-rhamnoside, isorhamnetin 3-*O*-glucoside, apigenin 7-*O*-apioglucoside [5], acicerone [6], mucronulactone, cajanine, maackianine [7], and canavanine [8]. A galactomannan was previously isolated in 15.8% yield (of the seed mass) from seeds of this species and had a galactose:mannose ratio of 1:1.33 [9]. However, its structure was not investigated in detail. The goal of our work was to isolate and characterize structurally galactomannan from *A. cicer* seeds.

A complex of water-soluble polysaccharides was isolated in 11.8% yield (of the seed mass) from *A. cicer* seeds. Precipitation by Fehling solution produced a polysaccharide fraction ACGm (5.9% yield of seed mass) that was a white fibrous powder and gave a viscous solution upon dissolution. Hydrolysis of ACGm produced galactose and mannose in a 1:1.39 ratio. The principal physicochemical properties of ACGm were $[\alpha]_D$ +71.9° (*c* 0.5, H_2O), [η] 925.5 mL/g (*c* 0.5, H_2O), molecular weight 1064 kDa. The IR spectrum of ACGm was similar to that of galactomanans [10]. All previously established parameters enabled us to assign the isolated polysaccharide to this class of biopolymers.

Periodate oxidation of ACGm consumed 1.42 mol of $NaIO_4$ per anhydro unit and released 0.41 mol of HCOOH. The Smith degradation products were glyerine and erythritol in a 1:1.35 ratio. These results were consistent with (1→4)- and (1→6)-type polysaccharide bonds in the studied polysaccharide.

Next galactomannan ACGm was methylated by the Ciucanu–Kerek method. The resulting permethylate was subjected to formolysis and hydrolysis. The hydrolysate contained 2,3,4,6-tetra-*O*-Me-Gal*p*, 2,3-di-*O*-Me-Man*p*, and 2,3,6-tri-*O*-Me-Man*p* in a 2.58:2.56:1 ratio. These results indicated that the main chain of ACGm consisted of mannnopyranose units with (1→4)-bonds that were substituted at the C-6 position by galactopyranose units.

The configurations of galactose and mannose were determined after CrO_3 oxidation of the acetylated galactomannan and subsequent hydrolysis of the oxidation products. The only monoside present in the hydrolysate was galactose, which argues for the α-configuration of its anomeric center and the β-configuration for mannose because it was not observed.

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

	δ_C, ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
α-Gal*p*-1→	99.01	68.61	69.12	69.97	71.76	62.08
→4-β-Man*p*-1→	101.08	70.15	72.04	77.83 78.07	76.64	62.24
→4,6-β-Man*p*-1→	99.69	71.17	72.31	78.07 78.24	74.88	67.43

Partial acid hydrolysis of ACGm produced galactose and mannose in addition to four oligosaccharides A-1, A-2, A-3, and A-4. These were isolated using gal chromatography over Sephadex G-10 and preparative PC. They were identified as mannobiose [β-D-mannopyranosyl-(1→4)-β-D-mannose], α-D-galactopyranosyl-(1→6)-β-D-mannose, mannotriose [β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannose], and mannotetraose [β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannose].

We recorded the ^{13}C NMR spectrum of partially depolymerized galactomannan ACGm-d, which had similar physicochemical properties but lower molecular weight (79.5 kDa). Table 1 gives the chemical shifts of C atoms in the ^{13}C NMR spectrum of ACGm-d and their interpretation.

The resonance for galactose C-1 was shifted to weak field (99.0 ppm). This was indicative of the α-configuration for its anomeric center. The chemical shifts of C-2–C-6 were similar to those of free α-D-galactopyranose. The position of the C-6 resonance (62.08 ppm) argued in favor of the pyranose ring. Thus, galactose in the studied galactomannan was present as single units of α-D-galactopyranose [11].

Mannose units typically had the β-configuration. This was determined from the chemical shift of C-5 of 76.6 ppm. Otherwise the resonances of this atom would have been located at stronger field [12]. The weak-field shifts of C-1, C-4, and C-6 relative to free mannopyranose of 6.18, 9.93, and 5.13 ppm, respectively, indicated that they were involved in bond formation. The chemical shift of C-6 of substituted mannose (67.43 ppm) suggested that the substituent had the α-configuration. The presence of a β-substituent would have caused a shift of greater than 70 ppm [13]. Furthermore, mannose had the pyranose form because the C-6 resonance was located at 62.24 ppm. The ratio of integrated intensities of C-1 of galactose and mannose was 1.37.

The resonances of mannopyranose C-4 were examined in order to determine the ratio of structural units in galactomannan ACGm [14]. The ratio of integrated intensities of resonances at 77.83, 77.07, and 78.24 ppm that corresponded to the presence in the polymeric chain of Man–Man blocks that were unsubstituted by galactose, the sum of two singly substituted blocks (Gal)Man–Man and Man–Man(Gal), and a doubly substituted block (Gal)Man–Man(Gal) was 1:1.06:3.29. Thus, the content of these blocks in ACGm was 18.7, 19.8, and 61.5%, respectively.

The investigation found that seeds of *A. cicer* contained a galactomannan, the main chain of which consisted of (1→4)-bonded β-D-mannopyranose units substituted 72% at the C-6 position by single α-D-galactopyranose units.

EXPERIMENTAL

Seeds of *A. cicer* L. were collected in August 2000 from plants introduced into the Central Siberian Botanical Garden, Siberian Branch, Russian Academy of Sciences (Novosibirsk).

High-performance TLC was performed on Sorbfil PTSKh-AF-V plates (Sorbpolimer); PC, on FN-8 chromatographic paper (Filtrak). The solvent systems were *i*-PrOH:$CHCl_3$:H_2O (7:4:1, double elution to 4 and 8 cm) (1); BuOH:Py:H_2O (15:30:20) (2), $CHCl_3$:MeOH (9:1) (3), PrOH:EtOH:H_2O (7:1:2.5) (4), and *i*-PrOH:H_2O (4:1, descending mode) (5). The developers were *p*-hydroxydiphenyl phosphate (1) and $KMnO_4$:$NaIO_4$:benzidine (2).

Standards were galactose and mannose (Acros Organics) and mannobiose (Carbosynth Lim.).

Optical rotation was measured on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant) in a 1-dm cuvette at 20°C. IR spectra were recorded on a Spectrum 100 IR-Fourier spectrometer (Perkin–Elmer) as films on KRS-5 plates in the range 4000–450 cm^{-1}. Spectrophotometric studies were performed on a UV-Vis-mini spectrophotometer (Shimadzu) 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 were recorded for 1% solutions in DMSO-d_6.

Water-soluble polysaccharides were isolated as described before [1] from *A. cicer* seeds (50 g) ground to a powder and defatted beforehand by hexane, $CHCl_3$, and acetone. The yield of water-soluble polysaccharides was 5.90 g.

Galactomannan (ACGm) was isolated as the copper complex using Fehling solution [15] Yield of ACGm, 2.95 g.

Total Hydrolysis. ACGm (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, developer 1). The quantitative monosaccharide composition was determined by HPTLC and densitometry.

Viscometric studies were carried out as before [16]. Molecular weights of polysaccharides were calculated based on the characteristic viscosities [17].

Periodate oxidation and Smith degradation were carried out as described earlier [1]. The hydrolysate after Smith degradation was analyzed by HPTLC (system 2, developer 2).

Methylation of ACGm was performed by the Ciucanu–Kerek method [18]; formolysis and hydrolysis of the permethylate, as before [19]. The hydrolysates were analyzed by HPTLC (system 3, developer 1) using comparison with authentic samples of methylated pyranoses.

Polysaccharides were oxidized by chromic anhydride after preliminary acetylation by the literature method [20].

Partial Hydrolysis. ACGm (1 g) was dissolved in water (100 mL), treated with HCl (20 mL, 5 M), and heated at 80°C for 4 h. The hydrolysate was neutralized over AV-17-8 anion-exchange resin (HCO_3^--form, Biolar), concentrated, and analyzed by HPTLC (system 4, developer 1).

Isolation of Oligosaccharides. The hydrolysate after partial hydrolysis was placed on a column of Sephadex G-10 (Pharmacia, 3 × 80 cm) and eluted with water (detection, phenol–H_2SO_4 method). The separation was monitored using HPTLC (system 4, developer 1). The contents of oligosaccharide fractions were rechromatographed by PC (system 5, developer 1) to isolate four substances A-1, A-2, A-3, and A-4. These were identified using chromatographic mobilities (HPTLC, system 4, developer 1), optical rotation, and methylation as β-D-mannopyranosyl-(1→4)-β-D-mannose, R_{Man} 0.53, $[\alpha]_D$ –8.4° (*c* 1.14, H_2O), 2,3,6-tri-*O*-Me-Man*p*:2,3,4,6-tetra-*O*-Me-Man*p* 1:1; α-D-galactopyranosyl-(1→6)-D-mannose, R_{Man} 0.44, $[\alpha]_D$ +116.3° (*c* 1.02, H_2O), 2,3,4-tri-*O*-Me-Man*p*:2,3,4,6-tetra-*O*-Me-Gal*p* 1:1; β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannose, R_{Man} 0.35, $[\alpha]_D$ –23.1° (*c* 0.83, H_2O), 2,3,6-tri-*O*-Me-Man*p*:2,3,4,6-tetra-*O*-Me-Man*p* 2:1; and β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannopyranosyl-(1→4)-β-D-mannose, R_{Man} 0.12, $[\alpha]_D$ –40.4° (*c* 0.65, H_2O), 2,3,6-tri-*O*-Me-Man*p*:2,3,4,6-tetra-*O*-Me-Man*p* 3:1.

Depolymerization of ACGm. ACGm (350 mg) was dissolved in water (60 mL), treated with HCl (5 mL, 1 M), heated at 100°C for 2 h, and centrifuged. The supernatant was precipitated with EtOH (95%, 1:4). The resulting precipitate (ACGm-d) was rinsed with EtOH (80%) and acetone and dried. Yield of ACGm-d, 230 mg (65.7% of ACGm mass). Gal:Man ratio, 1:1.38, $[\alpha]_D$ +72.2° (*c* 0.5, H_2O), [η] 72.8 mL/g (*c* 0.5, H_2O), molecular weight 79.5 kDa. The IR spectrum agreed with that of starting galactomannan ACGm.

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