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POLYSACCHARIDES OF Fabaceae. VI. GALACTOMANNANS FROM SEEDS OF *Astragalus alpinus* AND *A. tibetanus*

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Galactomannans with galactose:mannose ratios 1:1.48 and 1:1.33, $[\alpha]_D$ +67.9 and +76.4°, [η] 870.3 and 1337.1 mL/g, and molecular weights 999 and 1549 kDa, respectively, were isolated in 0.59 and 4.65% yields (of seed mass) from seeds of Astragalus alpinus *and* A. tibetanus *(Fabaceae). Physicochemical methods (CrO_3 oxidation; methylation–GC/MS; IR, NMR, and ^{13}C spectroscopy) found that the main polysaccharide chain consisted of 1,4-β-D-mannopyranose units substituted 67.5% (*A. alpinus*) and 75.2% (*A. tibetanus*) at the C-6 position by single α-D-galactopyranose units. The contents of mannobiose blocks Man–Man, (Gal)Man–Man/Man–Man(Gal), and (Gal)Man–Man(Gal) variously substituted with galactose were according to ^{13}C NMR spectroscopy 15.9, 55.5, and 28.6% in* A. alpinus *galactomannan and 9.9, 42.3, and 47.8% in* A. tibetanus *galactomannan.*

Keywords: *Astragalus alpinus*, *A. tibetanus*, Fabaceae, galactomannans, ^{13}C NMR spectroscopy.

We have continued research on galactomannans from seeds of the family Fabaceae [1–7]. It is currently known that galactomannans occur in seeds of 18 species of the genus *Astragalus* [1–3, 8–13]. This class of polysaccharides was found earlier in seeds of *A. alpinus* L. [alpine milkvetch; syn. *A. astragalinus* (Hook.) A. et D. Love; *A. grossheimianus* Sosn.; *A. salicetorum* Kom.] and *A. tibetanus* Benth. Ex Bunge (Tibetan milkvetch; syn. *A. laxmanii* Bge.). However, galactomannans from these species were not studied chemically. The goal of our work was to isolate and characterize the structures of galactomannans from seeds of *A. alpinus* and *A. tibetanus*.

Galactomannans were isolated as follows. Defatted seeds were extracted with H_2O in order to obtain fractions of water-soluble polysaccharides, from which precipitation by Fehling solution and regeneration produced samples of polysaccharides AAGm and ATGm in yields of 0.59 and 4.65% of the seed mass of *A. alpinus* and *A. tibetanus*, respectively. Both polysaccharides were soluble in H_2O to form highly viscous solutions with positive specific rotation (Table 1). Total hydrolysis produced galactose and mannose in 1:1.48 (AAGm) and 1:33 (ATGm) ratios. According to viscosimetry, the molecular weights (MWs) of AAGm and ATGm were 999 and 1549 kDa, respectively. The results and IR spectra [14] enabled the isolated polysaccharides to be classified as galactomannans.

The configurations of galactose and mannose in AAGm and ATGm were determined by CrO_3 oxidation of previously acetylated polysaccharides. It was found that the hydrolysates contained oxidation products of only galactose. This was possible if it had the α-configuration. The lack of mannose was possible if its anomeric centers had the β-configuration.

The nature of the monosaccharide bonds in AAGm and ATGm were determined using methylation with subsequent formolysis and hydrolysis of the permethylates and analysis of the decomposition products by GC/MS. The methylated monosaccharides 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 2.07:2.08:1 (AAGm) and 3.04:3.03:1 (ATGm) ratios. This was consistent with the presence in the main polysaccharide chain of (1→4)-bound mannopyranose units substituted at the C-6 position by single galactopyranose units. The degree of substitution was 67.5 for AAGm and 75.2% for ATGm. According to previous results for galactomannans of the genus *Astragalus*, the degree of substitution of the mannose chain can be from 61.4% (*A. sinicus*) [10] to 87% (*A. austrosibiricus*) [8].

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TABLE 1. Characteristics of Starting and Depolymerized Galactomannans from Seeds of *A. alpinus* and *A. tibetanus*

Galactomannan	Yield, %	$[\alpha]_D$, ° (*c* 1.0, H_2O)	[η], mL/g	MW, kDa	Monosaccharide composition, mol%		Methylated carbohydrate ratio			Contents of variously substituted mannose blocks, %		
					Gal	Man	1	2	3	4	5	6
AAGm	0.59[a]	+67.9	870.3	999	40.3	59.6	2.07	2.08	1.0	–	–	–
AAGm-d	53.5[b]	+68.2	77.9	85.3	40.6	59.3	–	–	–	15.9	55.5	28.6
ATGm	4.65[a]	+76.4	1337.1	1549	42.9	57.0	3.04	3.03	1.0	–	–	–
ATGm-d	60.1[c]	+75.9	95.4	104.6	42.8	57.1	–	–	–	9.9	42.3	47.8

[a]Of raw material mass; [b]of AAGm mass; [c]of ATGm mass.
1) 2,3,4,6-tetra-*O*-Me-Gal*p*; 2) 2,3-di-*O*-Me-Man*p*; 3) 2,3,6-tri-*O*-Me-Man*p*; 4) Man–Man; 5) (Gal)Man–Man/Man–Man(Gal); 6) (Gal)Man–Man(Gal).

TABLE 2. ^{13}C NMR Data of Depolymerized Galactomannans AAGm-d and ATGm-d, ppm

Monosaccharide unit	C-1	C-2	C-3	C-4	C-5	C-6
			AAGm-d			
α-Gal*p*-1→	99.47	69.10	70.27	70.88	72.13	62.30
→4-β-Man*p*-1→	101.83	71.36	73.34	76.50 77.02	74.88	61.22
→4,6-β-Man*p*-1→	100.69	71.36	73.34	77.02 77.98	73.61	67.40
			ATGm-d			
α-Gal*p*-1→	98.95	68.83	70.13	70.89	72.86	62.28
→4-β-Man*p*-1→	101.60	71.70	73.49	76.45 77.39	75.30	61.13
→4,6-β-Man*p*-1→	100.34	71.70	73.49	77.39 78.57	74.71	67.33

AAGm and ATGm were studied further by ^{13}C NMR spectroscopy of partially depolymerized polysaccharides (AAGm-d and ATGm-d, respectively) (Table 2), which were prepared using the literature method [15]. The resulting depolymerization products had similar physicochemical properties but lower MWs (Table 1). The ^{13}C NMR spectra of AAGm-d and ATGm-d contained sets of resonances for substituted and unsubstituted β-(1→4)-bonded mannnopyranose of the main chain and single α-galactopyranose side chains bonded at the C-6 position to mannose units. The spectra were similar to those of galactomannans of the family Fabaceae that were previously reported [1–3, 5–7, 16].

Information on the detailed structure of the galactomannan polymeric chains was obtained by studying the resonances of mannopyranose C-4. It was found for AAGm-d that the ratio of integrated intensities of resonances at 76.50, 77.02, and 77.98 ppm, which were due to the presence of a Man–Man mannobiose block unsubstituted by galactose, the sum of two singly substituted (Gal)Man–Man and Man–Man(Gal) blocks, and doubly substituted (Gal)Man–Man(Gal) blocks in GMGm, was 1:3.5:1.8. The resonances of substituted mannopyranoses in ATGm-d were situated at 76.45, 77.39, and 78.57 ppm. The ratio of integrated intensities was 1:4.3:4.8. Thus, mannobiose units singly and doubly substituted by galactose dominated the structures of *A. alpinus* and *A. tibetanus* galactomannans. This phenomenon was noted earlier for *A. cicer* [3], *A. danicus* [2], *A. lehmanianus* [13], and *A. sericeocanus* [1] and was probably a structural feature of the polymeric galactomannan chain of the genus *Astragalus* in general.

EXPERIMENTAL

Seeds of *A. alpinus* and *A. tibetanus* were collected from introduction sites in the Central Siberian Botanical Garden (CSBG, SB, RAS, Novosibirsk). The species were determined by Cand. Biol. Sci. D. V. Sandanov (IGEB, SB, RAS). Seed

samples were stored in the seed bank of the Department of Biologically Active Compounds, IGEB, SB, RAS (Nos. Fb/s-14/2-11/0604 and Fb/s-14/3-11/0605).

HPTLC was carried out on Sorbfil PTSKh-AF-V silica-gel plates (Imid Ltd.) using PrOH:$CHCl_3$:H_2O (7:4:1, system 1) (double development to 3.5 and 7 cm) with detection by *p*-hydroxydiphenylphosphate (1%). Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant) in a 1-dm cuvette at 20°C. IR spectra were recorded in KBr pellets on an FT-801 IR-Fourier spectrometer (Simeks). GC/MS of monosaccharide methyl ethers was performed on a 5973 N GC/MS (Agilent Techologies) with a 6890N mass-selective detector (Agilent Technologies) with a diffusion 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 from DMSO-d_6 solutions (1%). Galactose and mannose (Acros Organics) were used as standards.

Isolation of Galactomannans from Seeds of *A. alpinus* and *A. tibetanus*. Ground seeds of *A. alpinus* (82 g) were extracted in a Soxhlet apparatus successively by $CHCl_3$, EtOAc, and acetone. The remaining raw material was dried and extracted with H_2O (1:15, 3×) 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). The mixture was held for 10 h at 10°C in order to form the precipitate, which was centrifuged (6,000 g, 20 min); washed with EtOH (70–95%), acetone, and EtOAc; and then dried to afford water-soluble polysaccharides (WSPS) (1.12 g). Galactomannan was isolated by precipitation from an aqueous solution of WSPS (1%) using Fehling solution with subsequent regeneration [17], demineralization [7], and deproteination [18]. The yield of AAGm galactomannan was 484 mg. Seeds of *A. tibetanus* (54 g) afforded a WSPS fraction (5.63 g) and ATGm galactomannan (2.51 g).

Viscosimetric studies were performed as before [19]. MWs of galactomannans were calculated from the characteristic viscosity values [20].

Total Hydrolysis. The compounds (20 mg) were 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 GC/MS (as methyl ethers).

AAGm. Gal–Man 1:1.48. IR spectrum (ν, cm^{-1}): 688, 770, 798, 815, 871, 904, 975, 1025, 1078, 1150, 1221, 1306, 1372, 1417, 2918, 3417. **ATGm.** Gal—Man 1:1.33. IR spectrum (ν, cm^{-1}): 688, 769, 797, 814, 871, 902, 975, 1025, 1083, 1149, 1219, 1305, 1374, 1416, 2919, 3417.

Oxidation by CrO_3 of acetylated AAGm and ATGm was carried out as before [21]. The oxidation product was hydrolyzed and analyzed by HPTLC (system 1); **methylation** of polysaccharides used MeI and the literature method [22]; formolysis and hydrolysis of the permethylate, as described previously [23]. Hydrolysates were analyzed by GC/MS.

Depolymerization of AAGm (144 mg) and ATGm (168 mg) was carried out in HCl solution (1 M) by the literature method [15] and produced depolymerized AAGm-d (77 mg) and ATGm-d (101 mg). **AAGm-d.** Gal–Man 1:1.46. IR spectrum (ν, cm^{-1}): 694, 769, 796, 815, 870, 903, 975, 1024, 1077, 1151, 1222, 1307, 1379, 1417, 2895, 3420. **ATGm-d.** Gal–Man 1:1.33. IR spectrum (ν, cm^{-1}): 688, 769, 798, 814, 871, 902, 975, 1027, 1079, 1151, 1221, 1308, 1380, 1418, 2933, 3387.

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REFERENCES

1. D. N. Olennikov and A. V. Rokhin, *Chem. Nat. Comp.*, **44**, 685 (2008).
2. D. N. Olennikov and A. V. Rokhin, *Chem. Nat. Comp.*, **45**, 297 (2009).
3. D. N. Olennikov and A. V. Rokhin, *Chem. Nat. Comp.*, **46**, 165 (2010).
4. D. N. Olennikov, I. Yu. Selyutina, and A. V. Rokhin, *Chem. Nat. Comp.*, **46**, 673 (2010).
5. D. N. Olennikov and A. V. Rokhin, *Appl. Biochem. Microbiol.*, **46**, 113 (2010).
6. D. N. Olennikov and A. V. Rokhin, *Appl. Biochem. Microbiol.*, **46**, 444 (2010).
7. D. N. Olennikov and A. V. Rokhin, *Appl. Biochem. Microbiol.*, **46**, 540 (2010).
8. R. Ya. Plennik, *Sib. Ekol. Zh.*, **2**, 281 (2007).

9. I. E. Lobanova, O. V. Anulov, and V. D. Shcherbukhin, *Turczaninowia*, **10**, 72 (2007).
10. H. L .Tookey, R. L. Lohmar, I. A. Wolff, and Q. Jones, *J. Agric. Food Chem.*, **10**, 131 (1962).
11. O. V. Anulov, N. I. Smirnova, N. M. Mestechkina, I. A. Shreter, and V. D. Shcherbukhin, *Prikl. Biokhim. Mikrobiol.*, **31**, 645 (1995).
12. N. I. Smirnova, I. E. Lobanova, and O. V. Anulov, *Rastit. Resur.*, **33**, No. 4, 68 (1998).
13. N. M. Mestechkina, O. V. Anulov, N. I. Smirnova, and V. D. Shcherbukhin, *Appl. Biochem. Microbiol.*, **36**, 582 (2000).
14. K. Kato, M. Nita, and T. Mizuno, *Agric. Biol. Chem.*, **37**, 433 (1973).
15. S. Boziek, M. Izzard, and A. Morrison, *Carbohydr. Res.*, **93**, 279 (1981).
16. V. D. Shcherbukhin and O. V. Anulov, *Prikl. Biokhim. Mikrobiol.*, **35**, 257 (1999).
17. *Methods of Carbohydrate Chemistry* [in Russian], Moscow, 1967, p. 286.
18. A. A. Mohamed and P. Rayas-Duarte, *Cereal Chem.*, **72**, 648 (1995).
19. N. M. Mestechkina, O. V. Anulov, N. I. Smirnova, and V. D. Shcherbukhin, *Prikl. Biokhim. Mikrobiol.*, **32**, 656 (1996).
20. N. I. Smirnova and V. D. Shcherbukhin, *Prikl. Biokhim. Mikrobiol.*, **24**, 653 (1988).
21. O. Ishurd, A. Kermagi, F. Zgheel, M. Flefla, M. Elmabruk, W. Yalin, J. F. Kennedy, and P. Yuanjiang, *Carbohydr. Polym.*, **58**, 41 (2004).
22. I. Ciukanu and F. Kerek, *Carbohydr. Res.*, **131**, 209 (1984).
23. D. N. Olennikov and L. M. Tankhaeva, *Chem. Nat. Comp.*, **43**, 501 (2007).