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Lamiaceae CARBOHYDRATES. VII. GLUCOARABINOGALACTAN FROM *Panzerina lanata*

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In continuation of research on polysaccharides from plants of the family Lamiaceae [1–3], we studied water-soluble polysaccharides from the aerial part of *Panzerina lanata* (L.) Sojak.

The aerial part of *P. lanata* was collected in August 2008 near the village of Atsagat (Buryatiya, Russia). Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). Spectrophotometric studies were performed on a UV-Vis-mini spectrophotometer (Shimadzu). IR spectra were recorded from films on KRS-5 plates in the range 4000–450 cm^{-1} on a Spectrum 100 Fourier-IR spectrometer (Perkin–Elmer). ^{13}C NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz. Spectra were taken from solutions (1%) in DMSO-d_6. GC–MS analysis of methylated monosaccharides was carried out in a 5973 N GC–MS (Agilent Technologies) with a 6890N mass-selective detector (Agilent Technologies) with a diffusion pump and PH-Innowax capillary column (30 m/250 μm/0.50 μm), temperature gradient 150–250°C, heating rate 2°C/min, He carrier gas, flow rate 1 mL/min. Carbohydrate content was determined by the anthrone method [4]; acetyl groups, using hydroxylamine [5] and IR spectroscopy methods [6]. Total hydrolysis of polysaccharides was carried out in TFA (2 M, 120°C, 2 h). Quantitative monosaccharide composition was determined by HPTLC [1]. Gel chromatography was performed on Sephadex G-100 (1.5 × 90 cm, Pharmacia), NaCl (0.3%) eluent, flow rate 0.1 mL/min, detection of products by phenol-H_2SO_4 [7]. Oxidation of polysaccharides by CrO_3 was performed after preliminary acetylation by the literature method [8]. Periodate oxidation, Smith degradation, and deacetylation were carried out as before [3]. Methylation was carried out by the literature method [9] with monitoring of the process by IR spectroscopy. Then formolysis and hydrolysis of the permethylate [1] and analysis by GC–MS were performed.

Water-soluble polysaccharides were isolated from raw material (200 g) by preliminary extraction with EtOH (80%, 1:10, 100°C, 10 × 1.5 h). The pulp was treated successively with water (1:20) at 20 and 100°C until the reaction for carbohydrates was negative (phenol-H_2SO_4 method). Aqueous extracts were concentrated separately, demineralized over a column of KU-2-8 cation-exchanger (H^+-form, Reakhim, 50 × 300 mm, water eluent). Effluents were neutralized with AV-17-8 anion-exchanger (HCO_3^--form, Biolar), concentrated to 100 mL, precipitated with acetone (1:6), and centrifuged (3000 g, 15 min) after 1 h. The resulting precipitates were washed with acetone and dried to afford two fractions of water-soluble polysaccharides that were soluble in cold (PLW_C) and hot (PLW_H) water in 0.54 and 5.54% yields of raw material mass, respectively.

PLW_C. Positive reaction with iodine; $[\alpha]_D^{20}$ +48° (*c* 1.0, H_2O); Ara-Gal-Glc 1:3.3:24.7; traces of Man, Rha, Xyl. Gel chromatography (MW, kDa): 34.5, 71.3. IR spectrum (ν, cm^{-1}): 3345, 2891, 1447, 1416, 1331, 1210, 1169, 1094, 1021, 992, 929, 851, 762, 703, 598.

PLW_H. Negative reaction with iodine; $[\alpha]_D^{20}$ +67° (*c* 2.0, H_2O); Glc-Ara-Gal 1:1.7:4.5; traces of Man, Rha. Gel chromatography (MW, kDa): 28.4, 54.2, 75.4. IR spectrum (ν, cm^{-1}): 3409, 2935, 1740, 1441, 1370, 1330, 1233, 1146, 1102, 1023, 925, 852, 831, 761, 630, 535.

Fraction PLW_C was not investigated further. PLW_H was fractionated by stepwise precipitation with EtOH to produce 3 subfractions PLW_H-1 (0.15 g, c_{EtOH} = 23%); PLW_H-2 (2.74 g, c_{EtOH} = 48%), and PLW_H-3 (0.94 g, c_{EtOH} = 76%). Dominant subfraction PLW_H-2 was homogeneous according to gel chromatography.

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TABLE 1. ^{13}C NMR Spectra of PLW_H-2 and PLW_H-2′

Monosaccharide unit	^{13}C chemical shifts, ppm						
	C-1	C-2	C-3	C-4	C-5	C-6	acetate
			PLW_H-2				
→6-Gal*p*-*α*-1→	100.29	70.04	71.42	70.11	72.03	66.03	
		81.74	84.67				
Ara*p*-*β*-1→	100.83	69.53	69.83	70.52	63.83		20.72
		68.14	68.39				175.94
Glc*p*-*α*-1→	99.06	74.01	76.07	70.63	73.08	60.91	
			PLW_H-2′				
→6-Gal*p*-*α*-1→	100.27	70.01	71.39	70.09	71.98	66.31	
		81.80	84.70				
Ara*p*-*β*-1→	100.95	69.54	69.87	70.48	63.80		
Glc*p*-*α*-1→	99.10	72.77	74.42	70.75	73.12	61.24	

PLW_H-2. Negative reaction with iodine; $[\alpha]_D^{20}$ +69° (*c* 1.8, H_2O); Glc-Ara-Gal 1:1.5:4.0; MW 75.4 kDa. IR spectrum (ν, cm^{-1}): 3410, 2935, 1740, 1439, 1370, 1331, 1232, 1152, 1104, 1022, 921, 855, 829, 624, 534. Acetyl groups 12.30% (hydroxylamine method); 13.58% (IR spectrum).

Periodate oxidation of PLW_H-2 consumed 1.27 mol $NaIO_4$ per single anhydro unit and released 0.61 mol HCOOH. Smith degradation products contained arabinose and glucose in a 1:1.2 ratio in addition to glycerine. This indicated the presence of 1→2 and 1→6 bonds in the polysaccharide structure.

Deacetylation of PLW_H-2 produced **PLW_H-2′**, $[\alpha]_D^{20}$ +65° (*c* 1.2, H_2O), MW 61 kDa. IR spectrum (ν, cm^{-1}): 3461, 2957, 1430, 1373, 1335, 1146, 1111, 1020, 919, 853, 832, 620. Oxidation of PLW_H-2′ consumed 1.61 mol $NaIO_4$ and released 0.62 mol HCOOH per anhydro unit. Smith degradation did not produce hexoses in the hydrolysate.

Oxidation of PLW_H-2′ peracetate by CrO_3 produced in the hydrolysate of the oxidation product glucose and galactose in a 1:3.9 ratio. This indicated that they had the *α*-configuration. Arabinose, for which the *β*-configuration is characteristic, was not observed.

Formolysis and hydrolysis products of PLW_H-2′ permethylate contained (GC–MS) 2,3,4-tri-*O*-Me-Gal*p*, 3,4-di-*O*-Me-Gal*p*, 2,4-di-*O*-Me-Gal*p*, 2,3,4-tri-*O*-Me-Ara*p*, and 2,3,4,6-tetra-*O*-Me-Glc*p* in a 1.50:1.14:1.09:1.49:1 ratio and traces of 2,3,4,6-tetra-*O*-Me-Gal*p* and 4-*O*-Me-Gal*p*, i.e., the main chain of PLW_H-2′ was constructed of (1→6)-bonded galactopyranose, a part of which was substituted at C-2 and C-3 by single units of arabinopyranose and glucopyranose.

Next the structures of PLW_H-2 and PLW_H-2′ were studied using ^{13}C NMR spectroscopy (Table 1). The positions of the resonances for galactose and glucose C-1 in the spectrum of PLW_H-2 suggested that their anomeric centers had the *α*-configuration; arabinose, *β*-configuration. Substituted C-2, C-3, and C-6 of galactose in the main chain resonated at 81.74 ppm, 84.67, and 66.03 ppm, respectively; acetyls, 20.72 and 175.94. Arabinose C-3 gave two resonances due to the presence in PLW_H-2 of acetylated and nonacetylated units, the ratio of integrated intensities of which were 1:3.95, i.e., four of five arabinose units were acetylated. Acetylation of arabinose C-3 was confirmed by a second resonance for C-2 at 68.14 ppm due to the *β*-effect of acetylation (strong-field shift of the resonance for the neighboring atom) [4]. The glucose C-2 resonance shifted to weak field relative to that of free *α*-glucopyranose due to the *α*-effect of acetylation of C-3, which shifts the position of the resonance of the neighboring C atom to weak field [10].

The ^{13}C NMR spectrum of PLW_H-2′ was missing resonances for acetyl C atoms and those due to acetylation of arabinose units (68.14 and 68.39 ppm in PLW_H-2). Also, resonances of glucose C-2 and C-3 shifted to strong field, which was a consequence of deacetylation.

We found that the dominant polymer of water-soluble polysaccharides from the aerial part of *P. lanata* was an acetylated glucoarabinogalactan, the main chain of which was constructed of *α*-(1→6)-galactopyranose units containing on C-2 and C-3 single arabinopyranose and glucopyranose units that were in turn acetylated at the C-3 position.

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