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LAMIACEAE CARBOHYDRATES. IV. WATER-SOLUBLE POLYSACCHARIDES FROM *Scutellaria baicalensis*

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The composition of water-soluble polysaccharides from the aerial part of Scutellaria baicalensis *Georgi (Lamiaceae) was studied. It was found that the dominant polymers were WSPS′-1, WSPS′-2, and WSPS′-3, which were characterized preliminarily as partially acetylated glucoarabinogalactans, for which antioxidant activity was found. Two homoglucans of the starch type were minor components.*

Key words: *Scutellaria baicalensis* Georgi, Lamiaceae, glucoarabinogalactans, antioxidant activity.

We continued research on the carbohydrate components of plants from the familly Lamiaceae [1] by isolating and characterizing preliminarily water-soluble polysaccharides (WSPS) from the aerial part of *Scutellaria baicalensis* Georgi.

Lipophilic substances were removed from *S. baicalensis* raw material, which was then treated with ethanol (80%) to afford a fraction (10.36-11.39% of absolute dry raw material mass) that contained the free carbohydrates glucose, galactose, and saccharose.

WSPS were isolated from the remaining solid by extraction with hot water, concentration, and precipitation with ethanol to afford fraction WSPS, which was a colored substance with protein, phenol, and ash components (Table 1). The ash elements of WSPS included 26 elements, the main components of which were Ca, Mg, Na, P, and Si (Table 2). In general, the elemental composition of the WSPS was similar to that of the aerial part of *S. baicalensis* [2].

Removal of minerals and proteins from WSPS produced the complex WSPS′ in 18.33% yield (of WSPS mass) that had positive optical rotation and gave a weak color with iodine solution. Total hydrolysis produced galactose, arabinose, and glucose in a 3.2:1.3:1.0 ratio (Table 1). Fractional precipitation of WSPS′ by ethanol separated five components WSPS′-1, WSPS′-2, WSPS′-3, WSPS′-4, and WSPS′-5. Table 3 lists the principal physicochemical properties of them.

The dominant components WSPS′-1, WSPS′-2, and WSPS′-3 had positive optical rotation $[\alpha]_D^{20}$, did not react with iodine, and consisted of galactose, arabinose, and glucose in 4.6:1.5:1, 3.8:2.4:1, and 28.7:8.7:1 ratios, respectively. The weak reaction with iodine of the total complex was due to the presence of WSPS′-4 and WSPS′-5, which probably were starch-type glucans (only glucose was detected in the hydrolysate).

Gel chromatography showed that WSPS′-1, WSPS′-2, and WSPS′-3 were homogeneous with molecular weights (MW) 23, 27, and 35 kDa, respectively; WSPS′-4 and WSPS′-5 were heterogeneous and contained two substances each with MW 11 and 22 kDa in 14:7 and 3:5 ratios, respectively.

IR spectra of WSPS′-1, WSPS′-2, and WSPS′-3 differed from those of WSPS′-4 and WSPS′-5 (Table 3). In the range 700-1000 cm^{-1}, WSPS′-1, WSPS′-2, and WSPS′-3 exhibited absorption bands for α-bonds of three types that were due to ring vibrations analogous to dioxane vibrations (type 1, 914-917 cm^{-1}), deformation (equatorial) vibrations (type 2*a*, 834-835 cm^{-1}); and pyranose-ring vibrations (type 3, 762-765 cm^{-1}). Bands in the ranges 860-864 and 981-985 cm^{-1} were typical of arabino-3,6-galactans [3]. IR spectra of WSPS′-1, WSPS′-2, and WSPS′-3 also contained maxima for absorption of esters (1239-1264 and 1719-1738 cm^{-1}) that disappeared after treatment of the solutions with base. This was consistent with the presence of acetyls in the polymer structures, the content of which was 2.87-3.15% (Table 3).

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TABLE 1. Physicochemical Properties of WSPS and WSPS′

Component	Yield, %	Content, %				$[\alpha]_D^{20}$, ° (*c* 0.5, water)	Monosaccharide composition, mol		
		carbohydr.	phenols	ash	N		Ara	Gal	Glc
SBW	4.99*	21.46±0.71	20.79±0.62	34.25±1.37	5.54±0.14	+14	27.1	56.3	16.5
SBW′	18.33**	98.31±2.14		<0.2		+34	23.9	57.8	18.2

*Of absolute dry raw material mass; **of WSPS mass.

TABLE 2. Elemental Composition of WSPS, %

Ag	1.06×10^{-4}	Cu	1.29×10^{-3}	Na	0.44	Sn	3.91×10^{-4}
Al	0.15	Fe	0.14	Nb	3.97×10^{-3}	Ti	0.03
Ba	0.21	La	4.01×10^{-3}	Ni	3.92×10^{-4}	V	1.30×10^{-3}
Be	1.36×10^{-4}	Mg	6.60	P	5.29	Y	1.29×10^{-3}
Ca	13.34	Mn	0.09	Pb	1.33×10^{-3}	Zn	0.01
Cr	1.98×10^{-3}	Mo	3.95×10^{-4}	Si	1.05	Zr	6.67×10^{-2}

TABLE 3. Physicochemical Properties of WSPS′ Components

Substance	C_{EtOH}, %*	Yield, %**	$[\alpha]_D^{20}$, ° (*c* 1.0, water)	K_{Ac}, %***	Monosaccharide composition, mol %			ν_{max}, cm^{-1}
					Ara	Gal	Glc	
SBW′-1	10.8	31.4	+32	2.87	21.8	64.2	13.9	3383, 2959, 1738, 1427, 1330, 1241, 1103, 1051, 1144, 1016, 983, 916, 862, 835, 762
SBW′-2	19.4	13.3	+30	3.01	22.7	74.6	2.6	3382, 2959, 1738, 1423, 1332, 1265, 1105, 1051, 1145, 1016, 985, 914, 860, 835, 765
SBW′-3	52.6	48.9	+37	3.15	28.4	52.7	18.9	3393, 2943, 1719, 1440, 1332, 1239, 1106, 1023, 1139, 1011, 981, 917, 864, 834, 763
SBW′-4	68.6	1.4	-	-	-	-	99.9	3425, 2930, 1648, 1452, 1425, 1375, 1242, 1200, 1156, 1080, 1022, 927, 853, 757, 708
SBW′-5	81.2	0.9	-	-	-	-	99.9	3429, 2931, 1649, 1458, 1425, 1377, 1240, 1204, 1150, 1081, 1022, 930, 852, 761, 710
Starch (Fluka)****								3462, 2928, 1646, 1457, 1420, 1374, 1238, 1206, 1156, 1082, 1017, 927, 853, 764, 708

*Ethanol concentration at which precipitation occurs; **of WSPS′ mass; ***K_{Ac}, content of acetyl groups; ****given for comparison.

IR spectra of WSPS′-4 and WSPS′-5 were similar to those of α-(1→4)-glucan starch. Type 1 bands in the fingerprint region were shifted to 927-930 cm^{-1}; type 3 bands, to 757-761 cm^{-1}.

WSPS from the aerial part of *S. baicalensis* were characterized preliminarily as partially acetylated glucoarabinogalactans (WSPS′-1, WSPS′-2, WSPS′-3) and glucans (WSPS′-4 and WSPS′-5).

TABLE 4. Antiradical and Antioxidant Activity of *S. baicalensis* Polysaccharides

Substance	DPPH-method, IC_{50}, mg/mL	Preservation of β-carotene, % of initial				
		Concentration, μg/mL				
		300	240	180	120	60
SBW′-1	0.216	70.98	61.53	56.05	44.78	12.60
SBW′-2	0.206	63.85	55.59	49.92	42.29	42.12
SBW′-3	0.198	71.64	70.99	70.98	66.60	60.70
Ascorbic acid	0.021	100	100	95.15	82.33	74.21

Investigation of the antiradical activity of WSPS′-1, WSPS′-2, and WSPS′-3 found that all components were able to trap DPPH radicals. The most active was WSPS′-3, which in turn exhibited the best antioxidant activity for the peroxide destruction of β-carotene model (Table 4).

The accumulation dynamics of WSPS in the aerial part of *S. baicalensis* showed a gradual increase of content until flowering (start of vegetation, 1.84%; budding, 1.85%; flowering, 1.93%) and a decrease until the end of vegetation (fruiting, 0.95%; end of vegetation, 0.26%).

EXPERIMENTAL

The aerial part of *Scutellaria baicalensis* Georgi was collected in June-September 2006 in Chita District (Russia). Raw material collected during flowering was used after removal of flower stalks to isolate polysaccharides.

Isolation of Polysaccharides. Ground raw material (400 g) was treated in a Soxhlet apparatus with $CHCl_3$:C_2H_5OH (2:1) until fully extracted. The defatted raw material was extracted with ethanol (80%, 1:20, × 4). The extract was concentrated to an aqueous remainder and treated with $Pb(CH_3COO)_2$ (5%) and Na_2SO_4 (10%) solutions. The aqueous phase was purified over columns of Al_2O_3, polyamide, cation-exchanger (KU 2-8, H^+), and anion-exchanger (ASD-4-5p, OH^-) (in all instances with H_2O eluent). The effluents were concentrated to afford a fraction of free carbohydrates (29.56 g, 7.39%).

The remaining raw material was treated to remove alcohol-soluble components and extracted with water (1:20, 100°C, 1.5 h, × 5). The extract was concentrated to 100-150 mL, treated with ethanol (95%, 1:5), stirred, and left for 1 h at 3-5°C. The resulting precipitate was separated by centrifugation, washed with ethanol (95%), reprecipitated, and dried by solvent exchange to afford water-soluble polysaccharides (WSPS) (19.96 g, 4.99%).

The carbohydrate content was determined by the anthrone method [4]; phenols, by the Folin method [5]; N, by the Bradford method using Coumassi G250 (ZAO Sileks) [6]; ash, gravimetrically after ashing; acetyl content, by reaction with hydroxylamine [7]; and optical rotation, on a Coers polarimeter, l = 10 cm at 20°C. Spectrophotometric studies were carried out on a Cecil CE 2011 spectrophotometer in 10-mm quartz cuvettes. Elemental composition of WSPS ash residual was determined on a DFS-8 spectrograph. IR spectra were recorded on a Perkin—Elmer IR-Fourier spectrometer as films on KRS-5 plates.

Total hydrolysis of polysaccharides was performed using TFA (2 M) at 100°C for 6 h, after which the hydrolysate was treated with anion-exchanger AV-17-8 (HCO_3^--form) and concentrated to the minimal volume in vacuo at 40°C.

Quantitative monosaccharide composition was determined by HPTLC and densitometry as described previously [1].

Demineralization of WSPS was performed over cation-exchanger KU-2-8 (H^+-form); **deproteinization**, using pronases from *Streptomyces griseus* (KF.3.4.244, Sigma) by the literature method [8]. WSPS (18 g) produced demineralized and deproteinized complex WSPS′ (3.30 g).

Fractionation by Ethanol. WSPS′ (3 g) was dissolved in water (50 mL) and treated in portions with ethanol (95%) until the concentration was 80%. The resulting precipitates were centrifuged and dried by solvent exchange to afford WSPS′-1, WSPS′-2, WSPS′-3, WSPS′-4, and WSPS′-5 in yields of 942, 399, 1467, 42, and 27 mg, respectively.

Gel chromatography was performed over Sephadex G-100 (1.5 × 60 cm, Pharmacia, Uppsala) with elution by NaCl solution (0.3%) at flow rate 0.1 mL/min and yield detection by the anthrone method.

Deacetylation of WSPS′-1, WSPS′-2, and WSPS′-3. The substance (50 mg) was dissolved in water (20 mL), treated with NaOH solution (2 mL, 2 M), left at room temperature with constant stirring for 1 h, and neutralized with HCl (2 mL, 2 M). Gel filtration over Molselect G-25 (Reanal, 2 × 40 cm, water eluent) was used for desalting. Effluents containing polysaccharides were precipitated with acetone, dried, and analyzed by IR spectroscopy.

The antiradical activity of the polysaccharides was determined by the DPPH method [9]; antioxidant activity, by oxidation of β-carotene with hydrogen peroxide in the presence of DMSO [1].

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