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LAMIACEAE CARBOHYDRATES. V. STRUCTURE OF GLUCOARABINOGALACTAN FROM *Scutellaria baicalensis*

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Chemical and spectral methods were used to establish that polysaccharide WSPS′-3 isolated from the aerial part of Scutellaria baicalensis *Georgi (Lamiaceae) was an acetylated glucoarabinogalactan, the main chain of which consisted of α-(1→6)-bonded galactopyranose with side chains consisting of arabinopyranose and partially acetylated glucopyranose residues located on C-2 and C-3.*

Key words: *Scutellaria baicalensis* Georgi, Lamiaceae, glucoarabinogalactan.

In continuation of research on polysaccharides from the aerial part of *Scutellaria baicalensis* Georgi, we isolated and characterized preliminarily a heterogeneous fraction of water-soluble polysaccharides (WSPS) consisting of acetylated glucoarabinogalactans (WSPS′-1, WSPS′-2, WSPS′-3) and glucans (WSPS′-4 and WSPS′-5) [1]. The homogeneous polysaccharide WSPS′-3 was the dominant compound. Therefore, we studied it in detail.

WSPS′-3 was composed of glucose, arabinose, and galactose in a 1:1.5:2.8 ratio and had molecular weight (MW) 35 kDa. The content of acetyls was 3.15%. The IR spectrum was similar to that of glucoarabinogalactans observed in certain species of *Acantophyllum* [2, 3]. Periodate oxidation consumed 1.20 mol of IO_4^- per single sugar residue and released 0.50 mol of HCOOH. The Smith degradation products contained arabinose, glycerine, and traces of glucose (PC). This was consistent with the presence of 1→2 and 1→6 bonds between the monosaccharide residues of the polymer.

Next, WSPS′-3 was deacetylated by NaOH. The process was monitored by IR spectroscopy until the ester absorption band disappeared. This produced polymer WSPS′-3-1 in 97.5% yield of the WSPS′-3 mass.

Thermal analysis (TG, DSC) of WSPS′-3 and WSPS′-3-1 established that both polymers typically gave two peaks, an endothermic one near 90-110°C due to loss of water and an exothermic one near 250-260°C caused by more extensive thermal degradation (disintegration) of the polymer chains. The endo- and exothermic peaks shifted in the DSC thermograms of the starting polymer to higher temperatures of 104 and 257°C, respectively (Fig. 1). This was due to the effect of acetylation [4]. Analogous peaks for the deacetylated polymer WSPS′-3-1 were located at 91 and 253°C.

Periodate oxidation of WSPS′-3-1 consumed 1.26 mol IO_4^- per single anhydro unit and released 0.51 mol of HCOOH. Smith degradation produced arabinose and glycerine with no glucose. The increase in the amount of consumed periodate during oxidation of the deacetylated polymer and the disappearance of glucose in the hydrolysate after degradation suggested that the glucose C atoms were acetylated in starting WSPS′-3.

WSPS′-3-1 was methylated by the Ciucanu—Kerek method. Formolysis of the permethylate and hydrolysis formed 2,3,4-tri-*O*-Me-Gal*p*, 3,4-di-*O*-Me-Gal*p*, 2,4-di-*O*-Me-Gal*p*, 2,3,4,6-tetra-*O*-Me-Glc*p*, 2.3.4-tri-*O*-Me-Ara*p*, and traces of 2,3,4,6-tetra-*O*-Me-Gal*p* and 2,4-di-*O*-Me-Ara*p* (TLC). This indicated that the main chain of the polysaccharide was constructed of (1→6)-bonded galactopyranose residues. The presence of disubstituted galactose (3,4-di-*O*-Me-Gal*p* and 2,4-di-*O*-Me-Gal*p*) suggested the presence of branching at C-2 and C-3. The side chains were formed by single glucopyranose and arabinopyranose residues in addition to a small number of short chains of (1→3)-bonded arabinose, which gave rise to the traces of 2,4-di-*O*-Me-Ara*p*.

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TABLE 1. ^{13}C NMR Spectra of WSPS′-3, WSPS′-3-1, and WSPS′-3-2

Monosaccharide residue	^{13}C Chemical shifts, ppm						
	C-1	C-2	C-3	C-4	C-5	C-6	acetate
				WSPS′-3			
→6-Gal*p*-α-1 →	100.93	70.53	71.04	70.15	71.90	66.87	21.88, 176.03
Ara*p*-β-1 →	101.67	69.50	69.97	70.61	63.61		
Glc*p*-α-1 →	99.94	72.88	76.14	70.25	72.82	61.44	
				WSPS′-3-1			
→6-Gal*p*-α-1 →	100.82	69.07	70.63	70.24	71.93	67.17	
Ara*p*-β-1 →	101.41	69.57	70.03	70.73	63.54		
Glc*p*-α-1 →	100.03	72.50	74.39	70.44	72.93	61.75	
				WSPS′-3-2			
→6-Gal*p*-α-1 →	99.72	68.66	71.21	69.74	72.10	66.73	

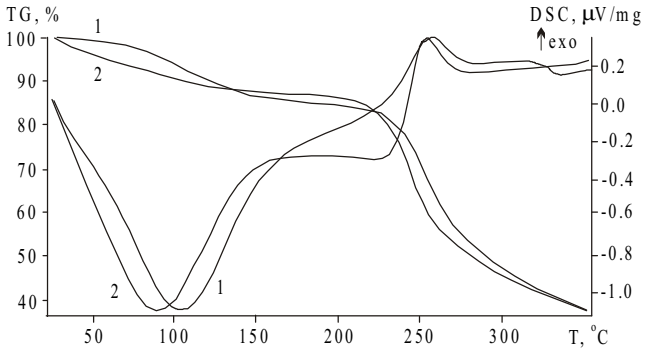

Fig. 1. TG and DSC thermograms of WSPS′-3 (1) and WSPS′-3-1 (2).

The hydrolysate of WSPS′-3-1 that was oxidized by chromic anhydride contained galactose and glucose. The proved that α-bonds were present. Arabinose was not detected because it has the β-configuration.

Structural features of WSPS′-3-1 were determined by partial hydrolysis, the products of which were the degraded polymer WSPS′-3-2 (56.2% yield of WSPS′-3-1 mass), glucose, arabinose, galactose, and galactobiose. The last was identified after preparative isolation (PC) by methylation.

The product of incomplete hydrolysis of WSPS′-3-2 was isolated and investigated. It was found that it contained only galactose, consumed 2.03 mol of periodate per mol of sugar residue, and released 0.98 mol of HCOOH. The hydrolysis product after Smith degradation was glycerine. The hydrolysate of WSPS′-3-2 permethylate contained 2,3,4-tri-*O*-Me-Gal*p* and traces of 2,3,4,6-tetra-*O*-Me-Gal*p*. The only product after CrO_3 oxidation was galactose (α-bond), i.e., this polymer was the linear segment of α-(1→6)-bonded galactose of the main chain.

^{13}C NMR spectra of WSPS′-3, WSPS′-3-1, and WSPS′-3-2 were also studied (Table 1).

Chemical shifts in the spectrum of WSPS′-3 for galactopyranose and glucopyranose at 100.93 and 99.94 ppm were consistent with their α-configuration whereas the β-configuration of arabinopyranose was proved by the location of the C-1 resonance at 101.67 ppm. Substituted C-2, C-3, and C-6 of galactopyranose resonated at 81.30, 83.63, and 66.87 ppm, respectively. The acetyls resonated at 21.88 and 176.03 ppm.

The general pattern of the ^{13}C NMR spectrum was retained after deacetylation except for the lack of resonances for acetyls and the shift of the C-3 resonance of glucopyranose compared with the starting polysaccharide. This confirmed that the glucose side chains were acetylated at the C-3 position.

The spectrum of WSPS′-3-2 contained six resonances corresponding to six α-(1→6)-bonded galactopyranose atoms. This is characteristic of linear polymers and confirmed the data for periodate oxidation and methylation.

Thus, the investigation established that the dominant polymer of the WSPS from the aerial part of *S. baicalensis* was WSPS′-3, which was a glucoarabinogalactan, the main chain of which was constructed of α-(1→6)-galactopyranose residues and had side branches of arabinose and partially acetylated glucose at C-2 and C-3.

EXPERIMENTAL

The isolation of WSPS and glucoarabinogalactan WSPS′-3 has been described by us previously [1]. PC was performed on Filtrak FN-2 and FN-14 paper; TLC, on Silufol plates (Kavalier); HPTLC, on Sorbfil PTSKh-AF-V plates (Sorbpolimer). The solvent systems were *i*-PrOH:H_2O (80:20, 1, descending mode); *i*-PrOH:$CHCl_3$:H_2O (7:4:1, 2, dual elution to 4 and 8 cm), $CHCl_3$:CH_3OH (9:1, 3). Developers were *p*-hydroxybiphenyl phosphate (1) and $KMnO_4$:$NaIO_4$:benzidine (2).

Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optical-Mechanical Plant) in a 1-dm cuvette at 20°C. IR spectra were recorded on a Spectrum 100 (Perkin—Elmer) Fourier spectrometer in a film on KRS-5 plates in the range 4000-450 cm^{-1}. Spectrophotometric studies were carried out on a Cecil CE 2011 spectrophotometer in quartz cuvettes (10-mm). Thermal analysis of polysaccharides was performed on a STA 449C (Netzsch) derivatograph in an Ar atmosphere in Pt—Rh crucibles in scan range 25-350°C at heating rate 10°C/min. ^{13}C NMR spectra were recorded on a VXR 500S (Varian) NMR spectrometer at operating frequency 125.7 MHz. Spectra were recorded for DMSO-d_6 solutions (1%) of the compounds.

Total hydrolysis of polysaccharides was performed in TFA (2 M) at 100°C for 6 h, after which the hydrolysate was treated with anion-exchanger AV-17-8 (HCO_3^--form), concentrated to the minimal volume in vacuo at 40°C, and analyzed by PC (system 1, developer 1) and HPTLC (system 2, developer 1). The quantitative monosaccharide composition was determined by HPTLC and densitometry as described earlier [5].

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 detection by phenol—H_2SO_4.

Periodate Oxidation and Smith Degradation. The studied polymer (50 mg) was dissolved in water (30 mL), treated with $NaIO_4$ solution (10 mL, 0.2 M), and left at 4°C. An aliquot of the solution was taken every 24 h and analyzed for IO_4^- content (spectrophotometry from the decrease of absorption at 223 nm [6]) and HCOOH (titration with NaOH solution, 0.01 M). The reaction was stopped after 10 d by adding ethyleneglycol (1 mL). Water (20 mL) and $NaBH_4$ (200 mg) were added. The solution was treated after 12 h with cation-exchanger KU-2-8 (H^+-form). The filtrate was concentrated to dryness in the presence of methanol. The dry solid was dissolved in H_2SO_4 (5 mL, 1 M) and heated to 100°C for 6 h. The hydrolysate was treated with anion-exchanger AV-17-8 (HCO_3^--form), concentrated to the minimal volume in vacuo at 40°C, and analyzed by PC (system 1, developer 2).

Oxidation by CrO_3 of polysaccharides was performed after preliminary acetylation by the literature method [7].

Deacetylation of WSPS′-3. WSPS′-3 (2 g) was dissolved in water (200 mL), treated with NaOH solution (20 mL, 2 M), left at room temperature with constant stirring for 1 h, neutralized with HCl solution (20 mL, 2 M), and placed on a column with Molselect G-25 (Reanal, 2 × 80 cm, water eluent). Effluents containing polysaccharides were precipitated with acetone and dried. Yield of deacetylated WSPS′-3-1, 1.95 g; $[\alpha]_D^{20}$ +32° (*c* 2.0, H_2O); MW 32 kDa. IR spectrum (ν, cm^{-1}): 3401, 2931, 1439, 1325, 1102, 1041, 1010, 980, 915, 864, 830.

Methylation of WSPS′-3-1. WSPS′-3-1 (200 mg) was methylated by the literature method [8] with monitoring by IR spectroscopy. Yield of permethylate, 158 mg. Then, formolysis and hydrolysis of the permethylate were carried out as described previously [5]. The hydrolysates were analyzed by TLC (system 3, developer 1) and compared with authentic samples of methylated pyranoses.

Partial Hydrolysis. WSPS′-3-1 (1.5 g) was dissolved in H_2SO_4 (20 mL, 0.2 M) and heated at 100°C for 5 min. The hydrolysate was treated with anion-exchanger AV-17-8 (HCO_3^--form) and precipitated with ethanol (95%, 1:5). The resulting precipitate (WSPS′-3-2) was centrifuged and dried. Yield of WSPS′-3-2, 843 mg; $[\alpha]_D^{20}$ +62° (*c* 1.5, H_2O); MW 11 kDa. IR spectrum (ν, cm^{-1}): 3383, 2930, 1448, 1321, 1100, 1040, 1002, 983, 917, 865, 827. Methylation, formolysis, hydrolysis, and TLC were performed as before. Yield of permethylate, 104 mg (from 180 g WSPS′-3-2).

The supernatant after removal of WSPS′-3-2 was concentrated and analyzed by PC (system 1, developer 1) and HPTLC (system 2, developer 1). Glucose, arabinose, galactose, and an oligosaccharide were detected.

The oligosaccharide was isolated by preparative PC (system 1) to afford a substance (42 mg) with $[\alpha]_D^{20}$ +140° (*c* 1.0, H_2O). Methylation by the literature method [8], formolysis, and hydrolysis of the permethylate produced 2,3,4,6-tetra-*O*-Me-Gal*p* and 2,3,4-tri-*O*-Me-Gal*p* in a 1:1 ratio (TLC). The results were compared with the literature [3] and indicated that the oligosaccharide was a galactobiose.

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