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Lamiaceae CARBOHYDRATES. VI. WATER-SOLUBLE POLYSACCHARIDES FROM *Lophanthus chinensis*

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The composition of water-soluble polysaccharides from the aerial part of Lophanthus chinensis *Benth. (Lamiaceae) was studied. The dominant polymer LCW_H-2, which was a partially acetylated glucoarabinogalactan, the main chain of which was constructed of α-(1→6)-bonded galactopyranose, was isolated and characterized. Atoms C-2 and C-3 of the main chain had branches of single glucopyranose and arabinopyranose units and short chains of (1→3)-bonded arabinose.*

Key words: *Lophanthus chinensis*, Lamiaceae, glucoarabinogalactan.

Lophanthus chinensis Benth. (Lamiaceae) is a Tibetan medicinal plant that is used to treat several diseases of the cardiovascular and central nervous systems that are combined under the common name "planetary diseases" (stroke, infarct, paralysis, etc.) [1]. Information on the chemical composition of this species is limited to a mention of the presence in it of essential oil [2]. The goal of our work was to investigate the composition of water-soluble polysaccharides of the aerial part of *L. chinensis*.

Polysaccharides were isolated from the aerial part of the plant by aqueous extraction (cold and hot) to produce two polysaccharide fractions LCW_C and LCW_H in yields of 0.97 and 4.79% (of raw material mass), respectively.

Fraction LCW_C gave a positive reaction with iodine; contained glucose, galactose, and arabinose in a 35.1:2.4:1.0 ratio and trace quantities of rhamnose and xylose, had positive rotation $[\alpha]_D^{20}$ +23° (*c* 1.0, H_2O), and was heterogeneous according to gel chromatography (MW 15-80 kDa). The IR spectrum (ν, cm^{-1}) exhibited absorption bands characteristic of starch-like α-glucans at 3461, 2930, 1452, 1418, 1367, 1238, 1206, 1152, 1080, 1016, 992, 927, 853, 763, 707, and 605.

LCW_H did not react with iodine solutions. Total hydrolysis produced galactose, arabinose, xylose, glucose, and rhamnose in a 45.8:33.7:10.1:9.4:1.0 ratio and positive rotation $[\alpha]_D^{20}$ +34° (*c* 2.0, H_2O). The IR spectrum (ν, cm^{-1}) exhibited bands at 3399, 2938, 1740, 1441, 1369, 1330, 1232, 1023, 1010, 980, 919, 865, 831, and 760. Gel chromatography showed that LCW_H was polydisperse. Therefore, fractionation by stepwise precipitation by ethanol was used to produce homogeneous components in five fractions, LCW_H-1–CW_H-5. The dominant fraction was LCW_H-2, which made up 19.6% of the LCW_H mass and was studied in detail.

LCW_H-2 was homogeneous with MW 67 kDa. It consisted of galactose, arabinose, and glucose in a 9.3:7.1:1.0 ratio with $[\alpha]_D^{20}$ +42° (*c* 1.8, H_2O). IR spectrum (ν, cm^{-1}): 3400, 2937, 1735, 1440, 1371, 1330, 1231, 1025, 1010, 982, 917, 864, 828, 762. It should be noted that ester absorption bands (1735 and 1231) in the IR spectrum of LCW_H-2 disappeared after treatment of the polymer with alkali. This indicated that the polysaccharide structure included acetyls. According to chemical analysis (hydroxylamine method), the degree of LCW_H-2 acetylation was 9.15%; according to IR spectroscopy, 9.47%. The overall pattern of the IR spectrum of LCW_H-2 was similar to that of glucoarabinogalactans from *Acantophyllum* spp. [3] and acetylated glucoarabinogalactan isolated previously from the aerial part of *Scutellaria baicalensis* [4].

Periodate oxidation of LCW_H-2 consumed 1.43 mol of $NaIO_4$ per single anhydro-unit and released 0.53 mol of HCOOH. The Smith degradation products contained arabinose, glucose, and glycerine, which indicated the presence of 1→2 and 1→6 bonds in the polysaccharide structure.

Next LCW_H-2 was deacetylated by alkali to produce polymer LCW_H-2′ in 82% yield of the starting mass.

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TABLE 1. ^{13}C NMR Data for LCW_H-2, LCW_H-2′, and LCW_H-2″

	^{13}C chemical shifts, ppm						
	C-1	C-2	C-3	C-4	C-5	C-6	acetate
				LCW_H-2			
→6-Gal*p*-*α*-1→	100.08	70.69	71.14	70.20	72.09	66.41	20.33
Ara*p*-*β*-1→	100.51	69.22	69.77	70.91	63.76	–	177.54
Glc*p*-*α*-1→	99.40	73.50	76.55	70.39	73.17	61.06	
				LCW_H-2′			
→6-Gal*p*-*α*-1→	100.03	70.51	70.95	70.14	72.11	66.48	
Ara*p*-*β*-1→	100.67	69.20	69.94	70.88	63.62	–	
Glc*p*-*α*-1→	99.77	73.67	74.21	70.29	73.14	61.12	
				LCW_H-2″			
→6-Gal*p*-*α*-1→	99.81	69.63	71.50	70.04	72.16	66.54	

Thermal analysis (TG, DSC) established that the derivatograms of the starting and deacetylated polymers were different and contained two main peaks each, and endothermic one due to dehydration (90-100°C) and an exothermic one (250-260°C) due to extensive structural disintegration. The main difference was a shift of the maxima for LCW_H-2 to higher temperatures, 95°C→105°C (endo) and 252°C→260°C (exo). This was explained by the influence of acetylation [5].

The consumption of $NaIO_4$ increased for periodate oxidation of LCW_H-2′ as compared with LCW_H-2. It reached 1.48 mol per single anhydro-unit. About 0.55 mol of HCOOH was released. The Smith degradation products were arabinose and glycerine. There was no glucose, confirming that its units were acetylated.

The main products in the hydrolysate of permethylated LCW_H-2′ were 2,3,4-tri-*O*-Me-Gal*p*, 3,4-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 2,4-di-*O*-Me-Gal*p*, 2,3,4,6-tetra-*O*-Me-Gal*p*, and 2,4-di-*O*-Me-Ara*p* (HPTLC). Therefore, the main chain of LCW_H-2′ consisted of (1→6)-bonded galactopyranose with branching at C-2 and C-3 (presence of 3,4-di-*O*-Me-Gal*p* and 2,4-di-*O*-Me-Gal*p*) formed by single glucopyranose and arabinopyranose units and short chains of (1→3)-bonded arabinose.

Chromic anhydride oxidation of LCW_H-2′ acetate gave hydrolysis products including galactose and glucose with the *α*-configuration. The absence of arabinose indicated that it had the *β*-configuration.

Partial hydrolysis of LCW_H-2′ produced glucose, arabinose, galactose, galactobiose, and a degraded polymer LCW_H-2″, which was isolated in 43.4% yield of the starting LCW_H-2′ mass.

Only galactose was detected in LCW_H-2″. Periodate oxidation established that the periodate consumption was 2.06 mol per anhydro-unit; the HCOOH release, 1.01 mol. The Smith degradation product was glycerine. Hydrolysis of the permethylate of LCW_H-2″ gave 2,3,4-tri-*O*-Me-Gal*p* and 2,3,4,6-tetra-*O*-Me-Gal*p* (traces). Chromic oxidation (hydrolysis product of the oxidized polymer was galactose), IR spectroscopy (ν, 915 cm^{-1}), and optical rotation $\{[\alpha]_D^{20}$ +68° (*c* 1.1, H_2O)$\}$ confirmed that the polymer structure contained an *α*-bond. Thus, LCW_H-2″ was a linear polysaccharide consisting of *α*-(1→6)-bonded galactopyranose and was a part of the main chain (core) of the starting polymer LCW_H-2.

LCW_H-2, LCW_H-2′, and LCW_H-2″ were further studied using ^{13}C NMR spectroscopy (Table 1).

The position of the resonances for C-1 of galactose and glucose at 100.08 and 99.40 ppm indicated that they had the *α*-configuration. Arabinose had the *β*-configuration of the anomeric center according to the resonance of C-1 at 100.51 ppm. Substituted atoms C-2, C-3, and C-6 of the galactose main chain typically resonated at 82.04, 85.71, and 66.41 ppm, respectively; the acetyls, at 20.33 and 177.54 ppm.

Deacetylation of LCW_H-2 affected the ^{13}C NMR spectrum of LCW_H-2′. Bands of acetyls disappeared. The resonance of glucopyranose C-3 shifted slightly. This confirmed that the starting polymer was acetylated at the corresponding C atoms of the side glucose units. The spectrum of LCW_H-2″ typically had six resonances that were assigned to C-atoms of an *α*-(1→6)-bonded galactose.

The results indicated that the pure polymer isolated from the aerial part of *Lophanthus chinensis* was a partially acetylated glucoarabinogalactan, the main chain of which contained α-(1→6)-galactopyranose with arabinopyranose and glucopyranose as side chains on C-2 and C-3.

EXPERIMENTAL

The aerial part of *Lophanthus chinensis* was collected in August 2007 near Atsagat (Buryatia).

HPTLC was performed on Sorbfil PTSKh-AF-V plates (Sorbpolimer) using solvent systems *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), and benzene:acetone (2:1) (3) and detectors *p*-hydroxydiphenylphosphate (1) and $KMnO_4$:$NaIO_4$:benzidine (2).

Optical rotation was determined on a SM-3 polarimeter (Zagorsk Optico-Mechanical Plant) in a 1-dm cuvette at 20°C. Spectrophotometric studiese were carried out on a UV-Vis-mini spectrophotometer (Shimadzu) in 10-mm quartz cuvettes. IR spectra in film on KRS-5 plates were recorded on a Spectrum 100 IR-Fourier spectrometer (Perkin—Elmer) in the range 4,000-450 cm^{-1}. Thermal analysis of polysaccharides was performed in a STA 449C derivatograph (Netzsch) under an Ar atmosphere in Pt—Rh crucibles with scanning in the range 25-350°C and heating at 10°C/min. ^{13}C NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz. Spectra were recorded for DMSO-d_6 solutions (1%). The content of acetyls was determined by spectrophotometry [6].

Isolation of Water-Soluble Polysaccharides. Ground raw material (300 g) was extracted in a Soxhlet extractor successively by hexane, $CHCl_3$, EtOAc, and EtOH (95%). The pulp was dried and treated successively with water (1:20 ratio) at 20°C until depleted. The extract was concentrated to about 200 mL, centrifuged (5,000 g, 20 min), and demineralized over a column of KU-2-8 cation-exchanger (H^+-form, Reakhim, 50 × 300 mm, water eluent). The effluent was neutralized with AV-17-8 anion-exchanger (HCO_3^--form, NPO Biolar), concentrated to 100 mL, precipitated with ethanol (95%, 1:6), and centrifuged after 1 h (3,000 g, 15 min). The resulting precipitate (LCW_C) was washed with ethanol and acetone and dried. Yield of LCW_C, 2.91 g (0.97% of raw material mass). Then raw material was treated with water (100°C, 1:30 ratio) until depleted. The extract was worked up as above to afford LCW_H, yield 14.37 g (4.79% of raw material mass).

Total Hydrolysis. Polysaccharide (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 methanol and analyzed by HPTLC (system 1, detector 1). The quantitative monosaccharide composition was determined by densitometry [7].

Gel chromatography was carried out over Sephadex G-100 (1.5 × 60 cm, Pharmacia, Uppsala) with elution by NaCl solution (0.3%) at 0.1 mL/min with detection by phenol:H_2SO_4 [8]. The standards were dextrans of molecular weights 2,000, 80, 50, and 10 kDa (all Pharmacia).

Fractionation by Stepwise Precipitation with Ethanol. LCW_H (10 g) was dissolved in water (150 mL) and treated with ethanol (95%) in portions. The resulting precipitates were centrifuged and dried to afford five fractions LCW_H-1 (0.65 g), LCW_H-2 (1.96 g), LCW_H-3 (0.83 g), LCW_H-4 (0.84 g), and LCW_H-5 (0.92 g).

Periodate Oxidation and Smith Degradation. The studied polysaccharide (100 mg) was dissolved in water (50 mL), treated with $NaIO_4$ solution (20 mL, 0.2 M), and stored at 4°C. Aliquots of the solution were taken every 24 h and analyzed for IO_4^- content by spectrophotometry [9] and for HCOOH by titration (0.01 M NaOH). The reaction was stopped after 10 d by adding ethyleneglycol (2 mL). The solution was treated with aqueous (30 mL) $NaBH_4$ (250 mg) and treated after 12 h with KU-2-8 cation-exchagner (H^+-form, 30 g). The filtrate was concentrated in the presence of methanol and then taken to dryness. The dry solid was dissolved in H_2SO_4 (5 mL, 1 M) and heated at 100°C for 6 h. The hydrolysate was treated with AV-17-8 anion-exchanger (HCO_3^--form), concentrated to the minimal volume in vacuo at 40°C, and analyzed by HPTLC (system 2, detector 2).

Oxidation by chromic anhydride was carried out after preliminary acetylation by the literature method [10].

Deacetylation of LCW_H-2. LCW_H-2 (1 g) was dissolved in water (50 mL), treated with NaOH solution (10 mL, 2 M), left at room temperature with constant stirring for 1 h, neutralized with HCl solution (10 mL, 2 M), and placed on a column of G-25 Molselect (Reanal, 2 × 80 cm, water eluent). Effluents with polysaccharides were precipitated with acetone and dried. Yield of deacetylated LCW_H-2′, 0.82 g, $[\alpha]_D^{20}$ +49° (*c* 1.7, H_2O)), MW 59 kDa. IR spectrum (ν, cm^{-1}): 3387, 2930, 1627, 1443, 1370, 1328, 1020, 1017, 979, 915, 866, 825, 759.

Methylation of LCW_H-2′ (200 mg) was carried out by the Ciucanu—Kerek method [11] with monitoring of the process by IR spectroscopy. Yield of permethylate, 173 mg. Next the permethylate underwent formolysis and hydrolysis [7]. The hydrolysates were analyzed by HPTLC (system 3, detector 1) by comparison with authentic samples of the methylated pyranoses.

Partial Hydrolysis. LCW_H-2′ (0.5 g) was dissolved in H_2SO_4 (10 mL, 0.2 M) and heated at 100°C for 5 min. The hydrolysate was treated with AV-17-8 anion-exchanger (HCO_3^--form) and precipitated with ethanol (95%, 1:5). The resulting precipitate (LCW_H-2″) was centrifuged and dried. Yield of LCW_H-2″, 217 mg, $[\alpha]_D^{20}$ +68° (*c* 1.1, H_2O), MW 9.5 kDa. IR spectrum (ν, cm^{-1}): 3389, 2962, 1440, 1324, 1099, 1041, 1004, 975, 915, 870. Methylation, formolysis, hydrolysis, and TLC (system 1, detector 1) were carried out as above. LCW_H-2″ (150 g) afforded the permethylate (94 mg).

The supernatant after removal of LCW_H-2″ was concentrated and analyzed by HPTLC (system 1, detector 1). Glucose, arabinose, galactose, and an oligosaccharide that was identified as galactobiose [4] were detected.

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