Chemistry of Natural Compounds, Vol. 47, No. 2, May, 2011 [*Russian original No. 2, March-April, 2011*]

CARBOHYDRATES FROM Lamiaceae. VIII. α-GLUCAN FROM *Scutellaria baicalensis* ROOTS

D. N. Olennikov,[1*] A. V. Stolbikova,[2] A. V. Rokhin,[3] and V. B. Khobrakova[1]

UDC 582.949.25:677.46

The homogeneous polysaccharide S_bRP-1″ with MW 110 kDa was isolated during an investigation of water-soluble polysaccharides from Scutellaria baicalensis *roots. Its aqueous solution gave a positive reaction with iodine and exhibited high specific rotation ($[\alpha]_D$ +188.0°). Chemical, chromatographic, and spectral methods established that S_bRP-1″ was a slightly branched glucan containing in the main chain α-(1→4)-glucopyranose units, 8.3% of which were branched at the C-6 atom with single α-glucopyranose units. Glucan S_bRP-1″ was shown to exhibit immunostimulating activity using an azathioprine model of immunosuppression.*

Keywords: *Scutellaria baicalensis*, Lamiaceae, α-4,6-glucan, immunostimulating activity.

We have continued our study of the carbohydrate components of plants from the family Lamiaceae [1]. Results from a study of the water-soluble polysaccharides (WSPS) from the aerial part of *Scutellaria baicalensis* Georgi were reported earlier [2]. Information on the polysaccharides from roots of this species has not been reported. The goal of the present work was to isolate and study the structure and biological activity of the dominant component of the WSPS complex from *S. baicalensis* roots.

WSPS were isolated from *S. baicalensis* roots using hot water extraction of raw material treated beforehand with EtOH (80%). This produced the fraction of total WSPS S_bRP in 2.53% yield of the air-dried raw material mass. Solutions of S_bRP gave a positive reaction with iodine and showed high specific rotation ($[\alpha]_D$ +190.4°). Hydrolysates of S_bRP contained glucose, galacturonic acid, galactose, arabinose, and mannose in a 33.5:3.0:2.4:1.8:1 ratio. Gel chromatography showed the S_bRP was heterogeneous, because of which it was fractionated using ion-exchange (DEAE-cellulose) and gel-permeation chromatography (Sephacryl 300-HR, Sephadex G-200). This isolated the homogeneous component S_bRP-1″ with molecular weight 110 kDa that eluted as a single symmetric peak by chromatography over Sephadex G-200.

The studies showed that S_bRP-1″ contained 97.2% carbohydrates; uronic acids and proteins were not observed. Aqueous solutions of the polymer reacted with iodine solution (λ_{max} 615 nm) and gave positive specific rotation ($[\alpha]_D$ +188.0°). Chromatography (HPTLC, GC/MS) of the hydrolysate of S_bRP-1″ detected only glucose. The IR spectrum of the polymer showed bands assigned to absorption by an α-bond (852 cm^{-1}) and bands characteristic of α-(1→4)-glucans (914, 1024, 1050, 1154 cm^{-1}) [3]. Oxidation of S_bRP-1″ acetate by chromic anhydride and hydrolysis of the oxidation product established the presence of glucose, which was possible for the α-configuration of the glucose units. Periodate oxidation of the polymer consumed 1.08 mol IO_4^- and released 0.08 mol HCOOH per single anhydro unit. Smith degradation produced glycerin and erythrite in a 1:12.06 ratio, which indicated the presence of (1→4)- and (1→6)-bonds.

The completely methylated polysaccharide was hydrolyzed. The decomposition products were analyzed as the aldoacetates by GC/MS. Three types of glucose derivatives were found. These were 1,5-diacetyl-2,3,4,6-tetra-*O*-Me-Glc*p*; 1,4,5-triacetyl-2,3,6-tri-*O*-Me-Glc*p*; and 1,4,5,6-tetraacetyl-2,3-di-*O*-Me-Glc*p* in a 1.05:12.21:1 ratio. The results confirmed that S_bRP-1″ contained in the main chain (1→4)-bonded glucopyranose, 8.3% of which were substituted at the C-6 position by single glucopyranose units. The slightly branched nature of the polymer was confirmed by the high values of the blue value, which was 0.392 and 0.320 at 610 and 680 nm, respectively [4].

1) Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences, 670047, Ulan-Ude, fax (3012) 43 30 34, e-mail: oldaniil@rambler.ru; 2) Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch, Russian Academy of Sciences, 664033, Irkutsk, fax (3952) 51 07 42, e-mail: alecsandrit@rambler.ru; 3) Irkutsk State University, 664033, Irkutsk, Russia, e-mail: rav@irk.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 176–179, March–April, 2011. Original article submitted August 1, 2010.

 0009-3130/11/4702-0190

TABLE 1. ^{13}C NMR Data for Glucan S_bRP-1″

Unit	^{13}C chemical shifts, ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
α-Glc*p*-1→	99.15	72.58	74.52	70.04	71.92	60.11
→4-α-Glc*p*-1→	99.15	72.45	73.16	77.83	71.92	60.11
→4,6-α-Glc*p*-1→	97.83	72.17	72.93	77.83	72.03	66.39

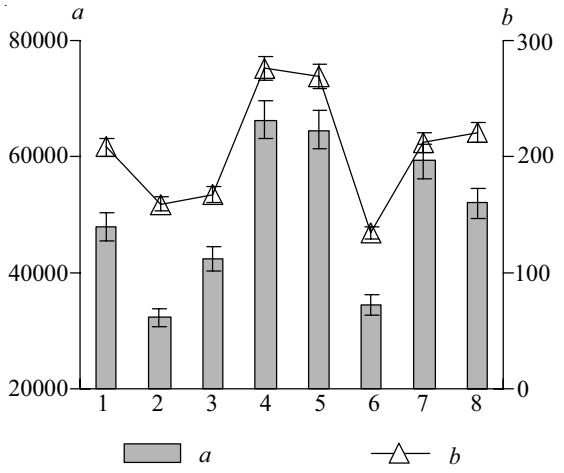

Fig. 1. Effect of glucan S_bRP-1″ on antibody formation: absolute number of spleen antibody-forming cells (a), number of antibody-forming cells per 10^6 spleenocytes (b). Groups: untreated (1), azathioprine (50 mg/kg) (2), azathioprine (50 mg/kg) + S_bRP-1″ (1, 10, and 100 mg/kg for 3, 4, and 5, respectively) (3-5), azathioprine (50 mg/kg) + rhamnogalacturonan MPP′-2 (1, 10, and 100 mg/kg for 6, 7, and 8, respectively) (6-8).

Glucan S_bRP-1″ was studied further using ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum showed resonances for glucopyranose side chains and unsubstituted and substituted glucopyranose of the glucan main chain (Table 1). The glucopyranose C-1 atoms typically gave resonances at weak field of 97.83 (substituted unit) and 99.15 ppm (unsubstituted unit) that indicated they had the α-configuration. A shift to weak field of the resonance for glucopyranose C-4 of the main chain (77.83 ppm) compared with that of free α-glucopyranose confirmed that it was involved in formation of a (1→4)-bond [5]. A resonance at 66.39 ppm was assigned to the C-6 atom of substituted glucopyranose units of the main chain. The magnitude of the shift confirmed that it had an α-substituent [6]. The positions of the remaining C resonances were consistent with previous data for other α-glucans and confirmed the structure of S_bRP-1″ as a branched α-4,6-glucan [3, 7–9].

An experimental pharmacological study of the effect of S_bRP-1″ on antibody-formation processes showed that the glucan could reduce the *in vivo* humoral immune response indices for an azathioprine immunosuppression model. Injection of azathioprine reduced the absolute number of antibody-forming cells (AFC) and the number of AFC per 10^6 spleenocytes (AFC/S) by 32.6 and 23.6%, respectively, compared with those of the untreated group (Fig .1).

The AFC and AFC/S indices increased reliably after administration of S_bRP-1″ during immunosuppression. The glucan exhibited the most pronounced immunostimulating activity at a dose of 10 mg/kg, at which the AFC and AFC/S indices increased by 105.2 and 73.6%, respectively, over the level of animals in the azathioprine group and by 38.2 and 32.7%, respectively, over the level of animals in the control group. The activity for the analogous dose of the reference drug, rhamnogalacturonan MPP′-2, which was isolated earlier from *Mentha piperita* leaves, was much less. The AFC and AFC/S indices increased by 82.9 and 33.3%, respectively, over the level of animals in the azathioprine group and by 23.2 and 1.9%, respectively, over the level of animals in the control group.

Thus, the studies showed that the dominant component of the WSPS complex from *S. baicalensis* roots was the homopolysaccharide S_bRP-1″, which was a slightly branched glucan, the main chain of which was constructed of α-(1→4)-glucopyranose units, 8.3% of which were substituted at the C-6 atom by single α-glucopyranose units. The isolated glucan attenuated the suppressive activity of azathioprine on the humoral component of the immune response index. The presence in *S. baicalensis* roots of a glucan with pronounced immunostimulating activity in addition to phenolic compounds explains the use of drugs from this plant raw material as immunostimulating agents.

EXPERIMENTAL

Roots of *S. baicalensis* were collected in Zabaikal Territory (Savvateevo; Sep. 28, 2005; 51°86′42″ N, 116°51′82″ E). The species was determined by Cand. Pharm. Sci. N. K. Chirikova (IGEB, SB, RAS). A specimen of the raw material is preserved in the Herbarium of the Department of Biologically Active Compounds, IGEB, SB, RAS (No. Lm/r-37/11-14/0905).

Gel chromatography was performed over Sephadex 300-HR (Sigma) and Sephadex G-200 (Pharmacia); ion-exchange chromatography, over cation-exchanger KU-2-8 (Reakhim) and DEAE-celluose (Reanal); HPTLC, on plates with Sorbfil PTSKh-AF-V silica gel (Imid Ltd.) using solvent systems PrOH:$CHCl_3$:H_2O (1, 7:4:1) (double development to heights 3.5 and 7 cm) and EtOAc:AcOH:MeOH:H_2O (2, 12:3:3:2) with detection by *p*-hydroxyphenylphosphate (1%). Spectrophotometric studies were carried out on a UV-Vis-mini spectrophotometer (Shimadzu). Optical rotation was measured on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). IR spectra were recorded as films on KRS-5 plates in the range 4000–650 cm^{-1} on a Spectrum 100 IR-Fourier spectrometer (Perkin–Elmer). GC/MS analysis was carried out in a 5973 N GC/MS (Agilent Technologies) with a 6890 N mass-selective detector (Agilent Technologies) with a diffusion pump and a PH-Innowax capillary column (30 m/250 μm/0.50 μm). ^{13}C NMR spectra were recorded from DMSO-d_6 solutions (1%) on a VXR 500 S NMR spectrometer (Varian) at operating frequency 125.7 MHz. Dialysis was performed in dialysis tubes with exclusion limit 1 kDa (Sigma). The content of carbohydrates was determined using the phenol-H_2SO_4 method [10]; of uronic acids, from the reaction with 3,5-dimethylphenol [11]; protein, by the Bradford method [12]; the blue value (B_v), by the Morrison–Lainglet method [13]. We used protease from *Streptomyces griseus* (EC 3.4.24.31, Fluka, CAS No. 9036-06-0, 6 U/mg) and azathioprine (Sigma).

Isolation and Fractionation of WSPS from *S. baicalensis*. Ground roots of *S. baicalensis* (500 g) were extracted by EtOH (80%, 1:10, 10 × 2 L) and then H_2O (1:12) on a boiling-water bath for 2 h (3×). The aqueous extracts were separated by centrifugation (6,000 g, 20 min) and combined. WSPS were precipitated by EtOH (95%, 1:5). The precipitate was centrifuged (6,000 g, 20 min), washed with EtOH (95%), resuspended in H_2O, and dialyzed against distilled H_2O. The undialyzed part was demineralized over cation-exchanger KU-2-8 (H^+-form, 3 × 40 cm, H_2O eluent) and deproteinized by protease [14] to afford the S_bRP fraction (12.65 g, 2.53% of raw material mass). Then, a weighed portion of S_bRP (11 g) was dissolved in H_2O (500 mL), placed on a column of DEAE-celluose (OH^--form, 4 × 70 cm), and eluted successively with H_2O and NaCl (0.3 and 0.7 M). The eluates were dialyzed and precipitated by acetone (1:4). The fractionation produced three fractions S_bRP-1 (H_2O, 8.316 g); S_bRP-2 (0.3 M NaCl, 0.0893 g), and S_bRP-3 (0.7 M NaCl, 0.484 g). Neutral fraction S_bRP-1 (8 g) was dissolved in H_2O (350 mL). The resulting solution was placed on a column of Sephacryl 300-HR (4 × 80 cm) and eluted by NaCl (0.1 M). The dominant fractions with molecular weight range 100–150 kDa were combined, dialyzed, and precipitated by acetone to afford fraction S_bRP-1′ (4.51 g). Then, a weighed portion of S_bRP-1′ (3 g) was dissolved in NaCl solution (100 mL, 0.3%). The resulting solution was placed on a column of Sephadex G-200 (3 × 90 cm) and eluted by NaCl (0.3%). Fractions containing the dominant component were combined, dialyzed, and precipitated by acetone (1:3) to afford fraction S_bRP-1″ (1.44 g).

S_bRP, $[\alpha]_D$ +190.4° (*c* 0.5, 1% NaOH). Glc, 80.4 mol%; Gal:Ara:Man, 2.4:1.8:1; uronic acids, 7.1 mol%. IR spectrum (ν, cm^{-1}): 606, 744, 834, 852, 890, 915, 958, 1019, 1050, 1078, 1099, 1146, 1226, 1331, 1415, 1643, 1730, 2934, 3399.

S_bRP-1″, $[\alpha]_D$ +188.0° (*c* 0.5, 1% NaOH). Molecular weight 110 kDa. UV spectrum of the complex with iodine (λ_{max}, nm): 615. B_V(λ): 0.392 (610 nm), 0.320 (680 nm). IR spectrum (ν, cm^{-1}): 612, 766, 852, 914, 1024, 1050, 1156, 1275, 1360, 1414, 2939, 3356.

Total Hydrolysis. The compound (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 MeOH and analyzed by HPTLC (solvent system 1) and GC/MS (as methyl ethers).

Oxidation by CrO_3 of acetylated S_bRP-1″ was carried out by the literature method [15]. The oxidation product was hydrolyzed and analyzed by GC/MS. Periodate oxidation and Smith degradation were performed as described earlier [7]; methylation of S_bRP-1″, by the methyliodide method [1]; formolysis and hydrolysis of the permethylate, as described previously [17]. The residue of methylated carbohydrates was treated with $NaBH_4$, acetylated by Ac_2O, and analyzed by GC/MS [18].

Immunostimulating Activity of S_bRP-1″. Experiments were conducted on CBA male mice (18–20 g). The activity of the agent was studied in untreated animals and those with immunodepression induced by azathioprine that was administered once per day for five days to the control group of animals at a peroral dose of 50 mg/kg. An aqueous solution of S_bRP-1″ was administered once per day for 14 days to the test group with azathioprine immunosuppression at peroral doses of 1, 10, and 100 mg/kg. The untreated group received water according to an analogous schedule. The condition of humoral immunity was estimated from the number of AFC according to Cunningham [19]. Mice were immunized with i.p. sheep erythrocytes at a dose of 2×10^8 cells/mouse. The magnitude of the immune response was estimated from the number of AFC per spleen and per 10^6 cells with nuclei at five days after immunization. The reference drug was rhamnogalacturonan MPP′-2 from *Mentha piperita* [17], which was administered analogously to S_bRP-1″.

ACKNOWLEDGMENT

The work was supported financially by the Lavrent'ev Award for Young Scientists, SB, RAS.

REFERENCES

1. D. N. Olennikov, A. V. Nazarova, A. V. Rokhin, L. M. Tankhaeva, and T. V. Kornopol′tseva, *Chem. Nat. Comp.*, **45**, 864, 300 (2009).
2. D. N. Olennikov, N. K. Chirikova, and L. M. Tankhaeva, *Chem. Nat. Comp.*, **44**, 556 (2008); D. N. Olennikov, A. V. Rokhin, and L. M .Tankhaeva, *Chem. Nat. Comp.*, **44**, 560 (2008).
3. S.-G. Li, D.-G. Wang, W. Tian, X.-X. Wang, J.-X. Zhao, Z. Liu, and R. Chen, *Carbohydr. Polym.*, **73**, 344 (2008).
4. U. K. Sengupta, A. K. Mukherjee, and K. K. Sengupta, *Colloid Polym. Sci.*, **208**, 32 (1966).
5. G. M. Lipkind, A. S. Shashkov, Y. A. Knirel, E. V. Vinogradov, and N. K. Kochetkov, *Carbohydr. Res.*, **175**, 59 (1988).
6. A. V. Egorov, N. M. Mestechkina, and V. D. Shcherbukhin, *Appl. Biochem. Microbiol.*, **39**, 398 (2003).
7. W. Cao, X.-Q. Li, L. Liu, M. Wang, H.-T. Fan, C. Li, Z. Lv, X. Wang, and Q. Mei, *Carbohydr. Res.*, **341**, 1870 (2006).
8. L. Shi, Y.-L. Fu, and K.-S. Chen, *Fitoterapia*, **78**, 298 (2007).
9. W. Ni. X. Zhang, H. Bi, J. Iteku, L. Ji, C. Sun, J. Fang, G. Tai, Y. Zhou, and J. Zhao, *Carbohydr. Res.*, **344**, 2512 (2009).
10. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
11. A. T. Usov, M. I. Bilan, and N. G. Klochkova, *Bot. Mar.*, **35**, 43 (1995).
12. M. M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).
13. W. R. Morrison and B. Laignelet, *J. Cereal Sci.*, **1**, 9 (1983).
14. A. A. Mohamed and P. Rayas-Duarte, *Cereal Chem.*, **72**, 648 (1995).
15. O. Ishurd, A. Kermagi, F. Zgheel, M. Flefla, M. Elmabruk, W. Yalin, J. F. Kennedy, and P. Yuanjiang, *Carbohydr. Polym.*, **58**, 41 (2004).
16. I. Ciukanu and F. Kerek, *Carbohydr. Res.*, **131**, 209 (1984).
17. D. N. Olennikov and L. M. Tankhaeva, *Chem. Nat. Comp.*, **43**, 501 (2007).
18. D. P. Sweet, S. H. Shapiro, and P. Albersheim, *Carbohydr. Res.*, **40**, 217 (1975).
19. A. J. Cunningham, *Nature*, **207**, 1106 (1965).