*Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November-December, 2011]*

# BASE-SOLUBLE POLYSACCHARIDES FROM SCLEROTIA OF *Inonotus obliquus*

**D. N. Olennikov,[1*] A. V. Rokhin,[2] and S. V. Agafonova[3]**

UDC 582.287.238:547.458.5

Polysaccharides of *Inonotus obliquus* (Pers.) Pil. (Hymenochaetaceae) have been the subject of many investigations [1]. Heteroglucans [2, 3], fucoglucomannan [3], and proteoglycans [4, 5] were observed previously in water-soluble exo- and endopolysaccharides from sclerotia, mycelium, and culture medium of *I. obliquus*. Base-soluble polysaccharides (BSPS) of this basidial fungus are insufficiently studied. Homoglucans that belong to the $\beta$-glucan class according to enzymatic analysis are known to be present [6]. The goal of the present work was to isolate and study the structure of BSPS from sclerotia of *I. obliquus*.

Sclerotia of *I. obliquus* were collected in the Pribaikal Region of Buryatiya Republic (Goryachinsk, Aug. 14, 2010; 52°98′84″N, 108°28′95″E). Collected raw material was cut into pieces, dried to constant mass at 60°C, and ground to particle size 0.5 mm. The species was defined by Cand. Pharm. Sci. G. V. Chekhirova (IGEB, SB, RAS). Samples of *I. obliquus* were preserved in the herbarium of IGEB, SB, RAS (No. HY/fb-42/03-12/0810).

BSPS were isolated by extracting dried and ground *I. obliquus* raw material (200 g) in a Soxhlet apparatus successively with hexane and $CHCl_3$. The defatted raw material was dried to remove completely the solvents and then extracted successively with EtOH (96 and 50%, 1:50 × 7, 120 min) and $H_2O$ on a boiling-water bath (1:20, 10×, 60 min). The raw material remaining after EtOH and $H_2O$ extractions was treated successively with KOH solution (5%) containing $NaBH_4$ (1%) at 20°C (1:10, 5×, 180 min) and KOH solution (15%) containing $NaBH_4$ (5%) at 90°C (1:10, 5×, 60 min).

The base extracts were neutralized with HCl and dialyzed against distilled $H_2O$ (48 h). The undialyzed part was dissolved in NaOH solution (5%), treated with $FeSO_4$ solution (5%) (1:2), and stored for 2 h. The colored precipitate was removed by centrifugation (6,000 g, 20 min). The decolorized supernatant was neutralized with HCl and again dialyzed against distilled $H_2O$ (48 h). The undialyzed part was lyophilized to produce two BSPS fractions **$I_o$P-1** (5% KOH, 20°C; 1.125 g) and **$I_o$P-2** (15% KOH, 90°C; 2.415 g).

**$I_o$P-1**, $[\alpha]_D$ –94.2° (*c* 0.5, 1% NaOH). Glc 92.7 mol%; Gal–Man–Xyl 2.4:2.1:1. IR spectrum ($\nu$, $cm^{-1}$): 3418, 2892, 1652, 1456, 1435, 1374, 1260, 1204, 1163, 1078, 1037, 997, 890 ($\beta$-bond). MW (content, %): >200 kDa, 7; 100–120 kDa, 84; 70–10 kDa, 9.

**$I_o$P-2**, $[\alpha]_D$ +57.1° (*c* 0.5, 1% NaOH). Glc 94.3 mol%; Gal–Man 1.7:1. IR spectrum ($\nu$, $cm^{-1}$): 3410, 2883, 1644, 1457, 1364, 1157, 1064, 1022, 928, 847 ($\alpha$-bond), 820. MW (content, %): >300 kDa, 2; 200–150 kDa, 79; 100–80 kDa, 9; <50 kDa, 10.

The physicochemical analyses indicated that fraction **$I_o$P-1** contained $\beta$-glucans as the principal components; **$I_o$P-2**, $\alpha$-glucans. The dominant polymers were isolated by gel chromatography. A weighed portion of **$I_o$P-1** (1 g) was dissolved in NaOH solution (50 mL, 0.2%), placed on a Sephacryl 300-HR column (Pharmacia, 3 × 60 cm), and eluted with NaOH solution (0.2 M). The dominant fractions in the MW range 100–150 kDa were combined, neutralized with HCl, dialyzed, and precipitated with acetone. Rechromatography under the same conditions produced component **$I_o$P-1′** (482 mg). Fraction **$I_o$P-2** was separated by dissolving a weighed portion (2 g) in NaOH solution (100 mL, 0.1%), chromatographing over a Sephacryl 300-HR column (4 × 80 cm), and eluting with NaOH solution (0.2 M). Fractions in the MW range 200-150 kDa were collected. The effluents were worked up as above. Rechromatography produced component **$I_o$P-2′** (1.477 g). According to HPLC, **$I_o$P-1′** and **$I_o$P-2′** were pure polymers of MW 110 and 175 kDa.

**$I_o$P-1′**, $[\alpha]_D$ –97.4° (*c* 0.3, 1% NaOH). Glc 98.4 mol%. IR spectrum ($\nu$, $cm^{-1}$): 3398, 2864, 1652, 1451, 1435, 1373, 1264, 1201, 1164, 1081, 1032, 992, 891 ($\beta$-bond). Oxidation by $CrO_3$ of acetylated derivative **$I_o$P-1′** did not produce hexoses,

1) Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences, 670047, Ulan-Ude, ul. Sakh′yanovoi, 6, fax: (3012) 43 30 34, e-mail: oldaniil@rambler.ru; 2) Irkutsk State University, 664033, Irkutsk, ul. Lermontova, 123, e-mail: rav@irk.ru; 3) Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch, Russian Academy of Sciences, 664033, Irkutsk, ul. Lermontova, 132, e-mail: agafonova@sifibr.irk.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 6, November–December, 2011, pp. 847–848. Original article submitted April 23, 2011.

0009-3130/12/4706-0971 ©2012 Springer Science+Business Media, Inc. 

which confirmed that *β*-bonds were present. Methylation and hydrolysis of the permethylate established the presence of 2,4,6-tri-*O*-Me-Glc*p* as the principal product and trace quantities of 2,3,4,6-tetra-*O*-Me-Glc*p* and 2,4-di-*O*-Me-Glc*p*. The $^{13}$C NMR spectrum of **I*o*P-1′** contained six resonances [103.72 (C-1), 84.84 (C-3), 73.67 (C-5), 71.97 (C-2), 70.15 (C-4), 62.12 ppm (C-6)], which was consistent with the *β*-configuration for the glucopyranose units (shift of the C-1 resonance to weak field) and the presence of a (1→3)-bond between them (shift of the C-3 resonance to weak field) [7]. The results allowed **I*o*P-1′** to be characterized as a linear *β*-(1→3)-glucan. The presence of **I*o*P-1′** caused the absorption maximum of a basic solution of Congo Red to shift by 15 nm. This indicated that the polymer macromolecules had a twisted configuration [8]. The quantitative content of *β*-glucans was determined by the method of Nitschke et al. [9] and was 3.12 ± 0.09% (of the air-dried raw material mass).

**I*o*P-2′**, $[\alpha]_D$ +59.4° (*c* 0.4, 1% NaOH). Glc 97.1 mol%. IR spectrum (ν, $cm^{-1}$): 3399, 2897, 1638, 1452, 1360, 1157, 1063, 1024, 931, 850 (*α*-bond), 822. The hydrolysate from $CrO_3$ oxidation of **I*o*P-2′** acetate contained glucose (*α*-bond). The principal hydrolysis product of **I*o*P-2′** was 2,4,6-tri-*O*-Me-Glc*p* with 2,3,4,6-tetra-*O*-Me-Glc*p* present in trace quantities. The $^{13}$C NMR spectrum was characterized by six resonances [99.82 (C-1), 83.15 (C-3), 72.92 (C-5), 71.83 (C-2), 71.02 (C-4), 61.94 (C-6)], which confirmed the *α*-configuration of the glucopyranose (shift of the C-1 resonance to strong field) and (1→3)-bonds in the main polymer chain (shift of the C-3 resonance to weak field) [10, 11]. Thus, polysaccharide **I*o*P-2′** was a linear *α*-(1→3)-glucan. Homoglucans of this type were not observed earlier in *I. obliquus*.

Dialysis was carried out in dialysis tubes with 1-kDa exclusion limit (Sigma). Spectrophotometric studies were performed on an SF-2000 spectrophotometer (OKB Spektr). Optical rotation was measured on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). IR spectra were recorded as films on ZnSe window-substrates on an FT-801 IR-Fourier spectrometer (Simeks) in the range 4000–600 $cm^{-1}$. Hydrolysis, methylation, and the reaction with Congo Red were conducted as before [12]. HPLC was carried out in a Summit liquid chromatograph (Dionex) using a TSK gel GMPx1 column (Supelco, 300 × 7.8 mm, 5 μm), $H_2O$ mobile phase, 1 mL/min flow rate, 20°C, and UVD 170S UV-detector at λ 190 nm. GC/MS analysis of monosaccharide alditolacetates was performed on a 5973N 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). $^{13}$C NMR spectra were recorded from 1% solutions in NaOD on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz.


## ACKNOWLEDGMENT

The work was supported financially by the Lavrent′evskii Competition of Young Scientists, SB, RAS.


## REFERENCES


1. W. Zheng, K. Miao, Y. Liu, Y. Zhao, M. Zhang, S. Pan, and Y. Dai, *Appl. Microbiol. Biotechnol.*, **87**, 1237 (2010).
2. H. Chen, M. Yan, J. Zhu, and X. Xu, *J. Ind. Microbiol. Biotechnol.*, **38**, 291 (2011).
3. Y. O. Kim, S. B. Han, H. W. Lee, H. J. Ahn, Y. D. Yoon, J. K. Jung, H. M. Kim, and C. S. Shin, *Life Sci.*, **77**, 2438 (2005).
4. M. Kim, Y.-S. Jeong, G. T. Chun, and Y.-S. Cha, *J. Korean Soc. Food Sci. Nutr.*, **38**, 667 (2009).
5. T. Mizuno, C. Zhuang, K. Abe, H. Okamoto, T. Kiho, S. Ukai, S. Leclerc, and L. Meijer, *Int. J. Med. Mushrooms*, **1**, 301 (1999).
6. S. J. Rhee, S. Y. Cho, K. M. Kim, D.-S. Cha, and H.-J. Park, *LWT – Food Sci. Technol.*, **41**, 545 (2008).
7. D. N. Olennikov, S. V. Agafonova, G. B. Borovskii, T. A. Penzina, and A. V. Rokhin, *Appl. Biochem. Microbiol.*, **45**, 626 (2009).
8. B. A. Stone and A. E. Clarke, *Chemistry and Biology of (1→3)-Glucans*, La Trobe University Press, Victoria, 2009.
9. J. Nitschke, H. Modick, E. Busch, R. Wantoch von Rekowski, H.-J. Altenbach, and H. Molleken, *Food Chem.*, **127**, 791 (2011).
10. D. N. Olennikov, S. V. Agafonova, A. V. Nazarova, and A. V. Rokhin, *Chem. Nat. Comp.*, **46**, 444 (2010).
11. X. F. Bao and J. N. Fang, *Chin. Chem. Lett.*, **12**, 967 (2001).
12. D. N. Olennikov, S. V. Agafonova, G. B. Borovskii, T. A. Penzina, and A. V. Rokhin, *Appl. Biochem. Microbiol.*, **45**, 536 (2009).