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BRIEF COMMUNICATIONS

MINOR GLUCANS FROM *Laetiporus sulphureus* BASIDIOCARPS

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We have continued research on basidiocarps of *Laetiporus sulphureus* (Bull.:Fr.) Murr grown by implantation under Irkutsk Oblast conditions [1–3]. Alkaline-soluble polysaccharides (ASPS) of this species were found to contain glucans, the dominant component of which was laetiglucan I, a linear β-(1→3)-glucan [3]. Herein we study the structures of minor glucans from *L. sulphureus*.

Basidiocarps of *L. sulphureus* (80 g) were processed as before in order to isolate ASPS [3]. Separation of the ASPS complex produced fractions LSA-1a (11.62 g, laetiglucan I), LSA-1b (1.80 g), and LSA-2 (0.99 g) [3]. LSA-1b contained two components, LSA-1b-1 and LSA-1b-2, which were isolated by preparative gel chromatography (Sephadex G-200, 3 × 80 cm, NaOH eluent, 0.01 M). LSA-1b (1.5 g) afforded LSA-1b-1 (1.06 g) and LSA-1b-2 (180 mg).

LSA-1b-1. MW 180 kDa. IR and ^{13}C NMR spectra and methylation agreed with those of laetiglucan I [3].

LSA-1b-2. MW 150 kDa, $[\alpha]_D$ –21° (*c* 0.25, 0.1 M NaOH). IR spectrum (ν, cm^{-1}): 3289, 2933, 1645, 1342, 1140, 1021, 922, 890 (β-bond), 819. Only glucose was observed after total hydrolysis. Periodate oxidation consumed 0.266 mol of IO_4^- per anhydro unit and released 0.131 mol of HCOOH. The hydrolysate after reduction and Smith degradation of the polysaccharide oxidized by periodate contained glycerine and glucose in a 1:6.7 mole ratio. Glycerine was released from terminal glucose units. The presence of glucose indicated that (1→3)-bonds stable to periodate were present. Hydrolysis of the acetylated derivative of LSA-1b-2 oxidized by CrO_3 did not produce hexoses. This was possible if its anomeric centers had the β-configuration. Methylation of LSA-1b-2 produced a hydrolysate with permethylate 2,3,4,6-tetra-*O*-Me-Glc*p*; 2,4,6-tri-*O*-Me-Glc*p*; and 2,4-di-*O*-Me-Glc*p* in a 1:5.69:1.02 ratio. This indicated that the structure of LSA-1b-2 was a polysaccharide, the main chain of which consisted of (1→3)-bound glucose units substituted 14.7% at the C-6 position with single glucose units. ^{13}C NMR data conformed that LSA-1b-2 was an O-6 branched β-(1→3)-glucan (Table 1) [4–6].

LSA-2 was demineralized and deproteinated before being studied. This produced fraction LSA-2′ (82% yield per LSA-2 mass). Low-molecular-weight impurities were removed from LSA-2′ over a column of Molselect G-25 (3 × 20 cm, water eluent) and then over Sephadex G-100 (2 × 60 cm, water eluent). The resulting LSA-2″ (534 mg) was a homogeneous polysaccharide.

LSA-2″. MW 110 kDa, $[\alpha]_D$ +211° (*c* 0.18, 0.1 M NaOH). IR spectrum (ν, cm^{-1}): 3447, 2932, 1382, 1274, 1155, 1048, 1018, 917, 841 (α-bond), 763, 611. The polymer consisted only of glucose and did not undergo periodate oxidation. The hydrolysate after oxidation of its peracetate by CrO_3 contained glucose. The hydrolysate after methylation of LSA-2′ contained 2,4,6-tri-*O*-Me-Glc*p* as the main product and traces of 2,3,4,6-tetra-*O*-Me-Glc*p*. The ^{13}C NMR spectrum of LSA-2″ showed six resonances. The position of the resonance for C-1 (100.51 ppm) corresponded with the α-configuration of the glucoses whereas the shift of the resonance of C-3 to weak field was due to glycosylation of this position, indicating a (1→3)-type bond (Table 1). The data enable LSA-2″ to be identified as a linear α-(1→3)-glucan [7, 8].

The studied polysaccharides LSA-1b-2 and LSA-2″ were called laetiglucans II and III and were the type of glucans first isolated from *L. sulphureus* basidiocarps.

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TABLE 1. ^{13}C NMR Spectra of LSA-1b-2 and LSA-2″

Carbohydrate unit	^{13}C chemical shifts, ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
			LSA-1b-2			
Glc*p*-β-1→	103.32	73.50	77.53	69.83	76.43	60.93
→3-Glc*p*-β-1→	102.51	72.21	86.14	68.19	76.43	60.93
→3,6-Glc*p*-β-1→	102.51	72.21	85.63	68.19	74.02	67.73
			LSA-2″			
→3-Glc*p*-α-1→	100.04	70.52	83.27	69.17	72.83	60.32

Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). Spectrophotometric studies were carried out on a UV-Vis-mini spectrophotometer (Shimadzu). IR spectra on KRS-5 plates were recorded on a Spectrum 100 IR-Fourier (Perkin–Elmer) spectrometer in the range 4000–450 cm^{-1}. ^{13}C NMR spectra were recorded on a VXR 500S (Varian) NMR spectrometer at operating frequency 125.7 MHz. Spectra were taken from DMSO-d_6 solution (1%). The monosaccharide composition was determined by GC/MS. GC/MS analysis of methylated monosaccharides was carried out on a 5973 N (Agilent Technologies) GC/MS with a 6890N mass-selective detector (Agilent Technologies), a diffusion pump, and PH-Innowax (30 m/250 μm/0.50 μm) capillary column. Total hydryolysis of polysaccharides used TFA (2 M, 120°C, 2 h); analytical gel chromatography, Sephadex G-200 (1.5 × 90 cm, Pharmacia), NaCl (0.3%) eluent, flow rate 0.1 mL/min, detection by phenol:H_2SO_4 method [9]; periodate oxidation, Smith degradation as described earlier [10]. Polysaccharides were oxidized by CrO_3 after preliminary acetylation by the literature method [11]; methylation, according to Ciucanu and Kerek [12] with monitoring of the process by IR spectroscopy followed by formolysis, hydrolysis of the permethylate [10], and analysis by GC/MS.

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