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Polysaccharides of Basidiomycetes. Alkali-Soluble Polysaccharides from the Mycelium of White Rot Fungus *Ganoderma lucidum* (Curt.: Fr.) P. Karst

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Abstract—Two polysaccharides were isolated from submergedly cultured mycelium of the basidiomycete *Ganoderma lucidum* by extraction with alkali followed by fractionation with Fehling reagent. The polysaccharides were shown to be a linear (1→3)-α-D-glucan and a highly branched xylomannan containing a backbone built up of (1→3)-linked α-D-mannopyranose residues, the majority of which are substituted at O-4 by single β-D-xylopyranose residues or by disaccharide fragments β-D-Man*p*-(1→3)-β-D-Xyl*p*-(1→. Polysaccharide structures were elucidated by NMR spectroscopy in combination with methylation analysis and periodate oxidation. An interesting feature of the xylomannan is the simultaneous presence of α-D-mannopyranose and β-D-mannopyranose residues, the first forming the backbone, and the second being the non-reducing terminal units of disaccharide side chains.

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Higher Basidiomycetes can be used as a rich source of antitumor and immune modulating polysaccharides [1-3], such as the widespread (1→3)-β-D-glucans, extensive literature being devoted to their chemical structure and biological properties [4-7]. One of the most known biologically active mushrooms, the white rot fungus *Ganoderma lucidum*, is a popular agent of Eastern folk medicine [8, 9]. Since early times in China, Korea, and Japan, it has been imbued with charm-like properties and used to treat many various diseases.

The antitumor activity of *G. lucidum* extracts is thought to be connected with two groups of substances – triterpenoids and polysaccharides [8-10]. Structural analysis of biologically active polysaccharides isolated from *G. lucidum* has been reported from various studies. Linear α-glucans [11, 12], branched β-glucans [13-15], and various more complex polysaccharides such as arabinoxyloglucan [16], glucuronoglucan [17], and branched heteropolysaccharides with a backbone built up of (1→4)-linked D-mannose [18] or D-galactose residues [19], as well as glycoproteins, where terminal non-reducing residues of α-L-fucose [20, 21] are a characteristic component of branched carbohydrate chains, are all described in the literature. All of these biopolymers have antitumor or immune modulating action to a greater or lesser degree, but the variety of their structures does not allow us to find trusted correlations between their structure and biological activity.

The abundance of different polysaccharides extracted from nominally one source can be explained in a few ways. First, the taxonomy of the *Ganoderma* genus is not sufficiently developed [22], and different species or even different strains of one species may substantially vary in polysaccharide composition. Second, the nature of the substrate on which the mushroom is growing in natural conditions or during the process of artificial cultivation may significantly influence the polysaccharide composition. Moreover, in different works different parts of the fungus have been used – fruit bodies, spores, mycelium, or culture liquid – and this is another factor leading to varying results.

From a practical point of view, a readily available and reproducible source for isolation of biologically active

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substance is needed. For this reason, a promising strain of *G. lucidum* whose taxonomic position is clear was chosen at the Gause Institute of New Antibiotics of the Russian Academy of Medical Sciences. A new accelerated method of its submerged culturing, more effective than previously known methods and allowing strict control of growing conditions, was developed [23]. Preliminary studies have shown that polysaccharide extracts of the mycelium have significant antitumor activity [24]. The aim of our study was to extract polysaccharides from this strain of *G. lucidum* and to identify their chemical structure. The structure of two polysaccharides – a glucan and a xylomannan – isolated from mycelium alkali extracts is described in this work.

MATERIALS AND METHODS

Growth and primary processing of mycelium. The mycelium of *G. lucidum* (strain 5 from the collection of the Laboratory of Biosynthesis of Biologically Active Compounds of the Gause Institute of New Antibiotics, Russian Academy of Medical Sciences), grown under previously described conditions [23], was used for the extraction of polysaccharides. The mycelium was separated from the culture medium by filtration through lavsan fabric, dried to constant weight (48 h at 36°C), and disintegrated with a household coffee-grinder to powder-like condition.

Dry powdered mycelium (30 g) was mixed with three portions of 300 ml mixture of methanol, chloroform, and water (4 : 2 : 1) at room temperature for a few days. Three hundred milliliters each of chloroform and water were added to combined extracts, and after separation of layers, the organic layer was evaporated under vacuum and the mixture of non-polar components of the mycelium was obtained as dark-colored viscous syrup (yield 10 g). The water–methanol layer was also evaporated to get a dry mixture of polar low-molecular-weight components of mycelium (yield 5 g).

Isolation of polysaccharides. Three hundred milliliters of water was added to fat-free mycelium, and the suspension was stirred with heating on a boiling water bath for 4 h. The extract was separated by centrifugation, and the rest of the mycelium was treated under the same conditions three more times. The aqueous extracts obtained were concentrated under vacuum, and polysaccharides from these extracts were sedimented by adding three volumes of acetone; four fractions of water-soluble polysaccharides with yields 1.48, 0.41, 0.24, and 0.15 g were obtained. The remainder of the mycelium was stirred with 100 ml 1 M NaOH for 4 h at room temperature, and the remaining mycelium was treated with alkali under the same conditions two more times. The alkaline extracts were dialyzed, insoluble in neutral water polysaccharides being precipitated. The precipitate was separated by centrifugation, neutral supernatant was lyophilized, and three fractions of alkali-extracted and water-soluble polysaccharides were obtained with yields 3.69, 0.20, and 0.05 g. The precipitates of water-insoluble polysaccharides were combined, dissolved in 1 M NaOH, and dialyzed, and the pellet formed was suspended in distilled water and lyophilized. Thus, the total preparation of alkali-soluble polysaccharides of mycelium was obtained (yield 1.24 g).

Fehling reagent was added dropwise to the solution of 1.2 g of this preparation in 50 ml of 1 M NaOH until full precipitation of copper complex. The precipitate was separated, washed with water, and mixed a few times with ethanol/HCl (5% concentrated HCl in ethanol); after that it was washed with acetone and dried under vacuum yielding xylomannan I (yield 0.54 g), $[\alpha]_D^{15}$ +68.8 (c 0.6; 1 M NaOH). After separation of the copper complex, the mother liquor was acidified with hydrochloric acid and the pellet formed was washed with acidified ethanol and acetone and dried under vacuum, yielding crude glucan (yield 0.54 g).

Smith degradation of polysaccharides. Xylomannan I (0.2 g) was suspended in 50 ml 0.02 M $NaIO_4$ aqueous solution and kept in darkness for 5 days at room temperature (until the termination of periodate consumption monitored by the optical density change at 305 nm). Ethylene glycol (0.5 ml) was added to the suspension, it was dialyzed, 50 mg of $NaBH_4$ was added, it was left overnight, then concentrated HCl was added to reach 0.5 M, the sample left for 8 h at room temperature, and then it was dialyzed and lyophilized yielding xylomannan II (yield 0.1 g), $[\alpha]_D^{15}$ +78.4 (c 0.5; 1 M NaOH). Purified glucan (yield 0.12 g), $[\alpha]_D^{20}$ +227.7 (c 1.0; 1 M NaOH), was obtained by a similar processing of the crude glucan preparation (0.2 g).

Polysaccharide methylation. Polysaccharide (5 mg) was dissolved in absolute dimethyl sulfoxide (1 ml), fine-grained NaOH (30-40 mg) was added, and then CH_3I (0.5 ml) was added while mixing. The reaction mixture was kept for 1 h, then chloroform (5 ml) was added, washed a few times with water, then the chloroform solution was evaporated and the residue was hydrolyzed by heating with 2 M trifluoroacetic acid (1 ml) at 100°C for 8 h. Acid was removed by evaporation with ethanol, and the resulting methylated derivatives of monosaccharides were converted into alditol acetates and identified using chromato-mass-spectrometry [25].

Quantitative assay of monosaccharides. Neutral monosaccharides were identified after polysaccharide hydrolysis (2 M CF_3COOH, 8 h at 100°C) by GLC in the form of alditol acetates [26] with *myo*-inositol acetate as an internal standard. GLC was done on a Hewlett-Packard 5890A chromatograph (USA) supplied with HP-1MS capillary column and flame-ionization detector with temperature programming from 160 to 290°C at a rate of 7°C/min (using nitrogen as carrier gas). Data were analyzed with the Multichrome 1.5 computer program.

Identification of D-mannose. Mannose absolute configuration was determined by a known method [27] using GLC of 1-deoxy-1-(L-α-methylbenzylamino)-D-mannitol acetate obtained by reductive amination of the hydrolysis products of xylomannan I with L-α-methylbenzylamine.

Spectroscopic and polarimetric measurements. NMR spectra were obtained using a Bruker DRX-500 spectrometer (Germany) at 303 K. Polysaccharide samples (20-30 mg) were suspended in D_2O (1 ml), then NaOH was added to the concentration 0.5-1.0 M and stirred until total dissolution of the polysaccharides. The solutions were lyophilized, the residues were dissolved in 99.96% D_2O (1 ml) and supplemented with sodium salt of 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid (internal standard, δ_H 0), and the resulting solutions were used to register spectra. Spectral data were analyzed with the XWINNMR 3.6 program (Bruker). The parameters used for two-dimensional NMR spectra were described earlier [28]. Additional data: TOCSY experiment was done with a mixing time (MLEV17 spin-lock) 200 msec; ROESY experiment with a mixing time (ROE spin-lock) 200 msec; NOESY experiment with a mixing time 100 msec. The HMBC experiment was done with 60-msec delay for the evolution of distant constants of spin–spin interactions.

The optical activity of polysaccharide solutions was determined on a PU-7 automatic polarimeter (Russia).

RESULTS AND DISCUSSION

The conditions of submerged cultivation of *G. lucidum* and preparation of dry mycelium were described earlier in detail [23]. The traditional scheme of stepwise extraction [29] with some modifications was used for polysaccharide isolation. Powdered mycelium was first treated with homogenous mixture of methanol, chloroform, and water (4 : 2 : 1), which dissolves almost all polar and non-polar low-molecular-weight substances but does not dissolve biopolymers [30]. After dilution of the extract with water, separation of layers, and evaporation of the organic layer, it was determined that mass of the non-polar low-molecular-weight substances is one third of the initial biomass. Defatted mycelium was processed a few times with hot water to extract water-soluble polysaccharides. Hydrolysis of the resulting polysaccharide fractions produced rhamnose, fucose, xylose, mannose, glucose, and galactose in different proportions. The structure and biological activity of water-soluble polysaccharides will be studied in the future. The remaining mycelium after water extraction was processed with 1 M NaOH at room temperature. After neutralization or removing alkali by dialysis, polysaccharides insoluble in water at neutral pH values were precipitated. This fraction was purified by re-precipitation from alkaline solution, and the total preparation of alkali-soluble polysaccharides was obtained with a yield of 4% of the initial

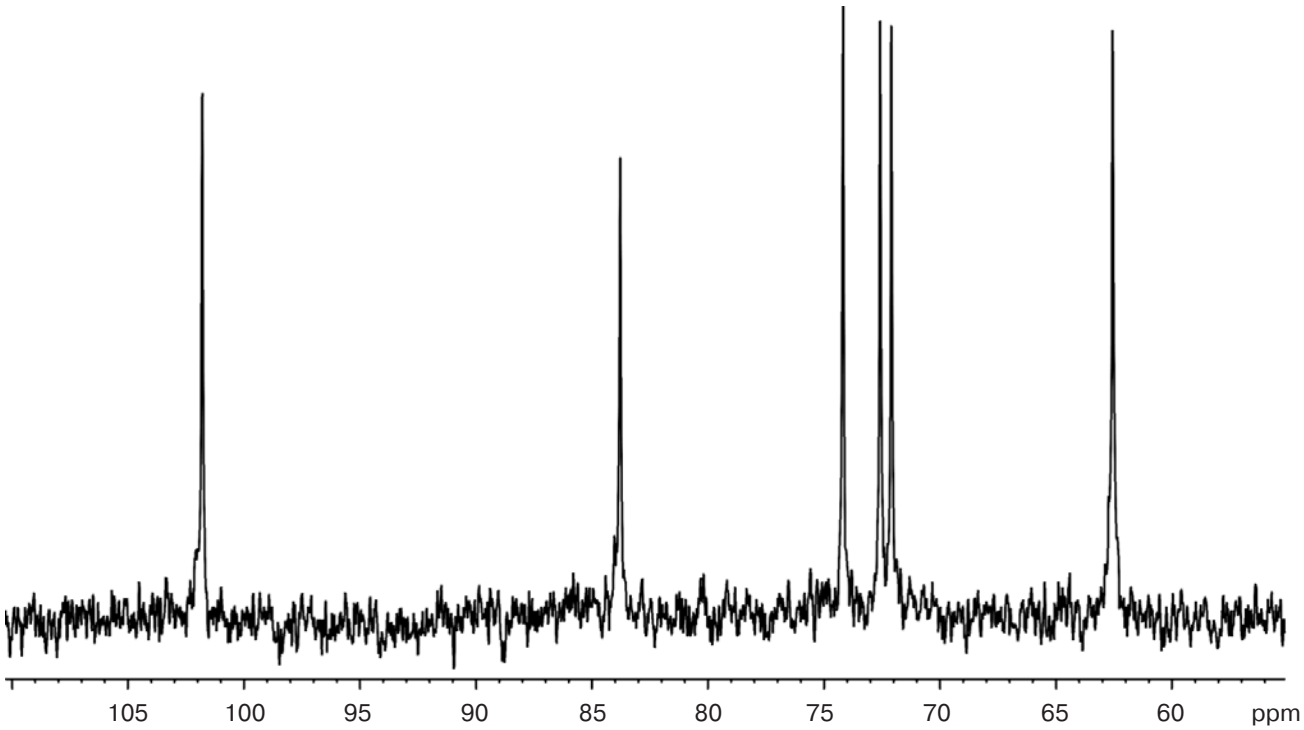


Fig. 1. ^{13}C-NMR spectrum of (1→3)-α-D-glucopyranan from *G. lucidum* (for signal assignment, see table).

Position of signals in NMR spectra of xylomannans I and II and glucan

Monosaccharide residue	Chemical shifts in ^{13}C- (internal standard acetone, δ_C 31.45) and ^{1}H-NMR spectra (internal standard TSP, δ_H 0.0)					
	C-1 H-1	C-2 H-2	C-3 H-3	C-4 H-4	C-5 H-5,5'	C-6 H-6,6'
Xylomannan II						
→3)-α-D-Man*p*-(1→ (**A**)	103.6*, 103.4** 5.13*, 5.16**	71.1 4.24	79.7 4.02	67.6 3.78	74.9 3.82	62.5 3.92, 3.78
→3)-α-D-Man*p*-(1→ (**B**) 4) ↑	103.4*, 103.1** 5.16*, 5.18**	71.1 4.20	76.8 4.14	76.4 3.99	74.1 3.93	61.8 3.92, 3.84
β-D-Xyl*p*-(1 (**C**)	105.2 4.42	75.1 3.28	77.2 3.45	70.7 3.64	66.6 3.96, 3.29	
Xylomannan I						
→3)-α-D-Man*p*-(1→ (**A**)	103.6*, 103.4** 5.13*, 5.15**	71.1 4.24	80.0 4.02	67.7 3.84	74.9 3.82	62.5 3.92, 3.78
→3)-α-D-Man*p*-(1→ (**B**) 4) ↑	103.1*, 102.9** 5.18*, 5.20**	71.1 4.18	76.8 4.12	76.7 4.04	74.1 3.93	61.8 3.98, 3.93
β-D-Xyl*p*-(1 (**C**)	106.0 4.42	75.4 3.28	78.0 3.43	71.1 3.63	67.1 3.94, 3.28	
→3)-β-D-Xyl*p*-(1→ (**D**)	106.3 4.44	75.1 4.41	86.4 3.62	69.8 3.73	66.9 3.97, 3.32	
β-D-Man*p*-(1→ (**E**)	102.0 4.93	72.0 4.15	74.6 3.67	68.4 3.59	78.0 3.44	62.7 3.96, 3.75
Glucan						
→3)-α-D-Glc*p*-(1→	101.8 5.32	72.6 3.64	83.8 3.88	72.1 3.54	74.2 4.02	62.6 3.88, 3.70
α-D-Glc*p*-(1→	5.28	3.53	3.72	3.35	3.95	3.88, 3.72

* Residues glycosylating A.
** Residues glycosylating B.

mycelium. In the hydrolysis products of this preparation we identified fucose, xylose, mannose, and glucose in molar proportion 1 : 13 : 9 : 15.

To evaluate homogeneity of the preparation, its reaction towards Fehling reagent was studied [31]. It was found that about half of the substance is precipitated by this reagent from alkali solution as a copper complex, while the remained stays in solution even at with excess reagent. After removing inorganic salts from the solution, we obtained polysaccharide preparation that gave practically only glucose during hydrolysis. In its ^{13}C-NMR spectrum, we observed signals specific for linear (1→3)-linked α-glucan [12, 32] together with a few signals of low intensity that we could not interpret. Considering that (1→3)-glucan is virtually inert to periodate oxidation [32], we used for its final purification the Smith degradation procedure [33] (oxidation by sodium periodate with subsequent reduction with sodium borohydride and mild acid hydrolysis). The resulting polysaccharide gave a perfect ^{13}C-NMR spectrum of (1→3)-α-glucopyranan (Fig. 1), whose signal composition differed only slightly from that described in literature spectra of similar polysaccharides [12, 32]. This can be explained by the differences in instrumental conditions. Signals assignment in this spectrum (table) was additionally confirmed using two-dimensional spectroscopy. High positive specific rotation did not leave any doubt that the polysaccharide consisted of D-glucose residues. The glucan structure was also analyzed by methylation, as a result of which only 2,4,6-tri-O-methyl- and 2,3,4,6-tetra-O-methyl-derivatives of glucose were obtained in proportion ~ 10 : 1. This corresponds to quite short linear (1→3)-linked chains of glucose residues. Terminal glucose residues were not identified in the ^{13}C-NMR spectrum, but the signals of these residues were found in the proton spectrum (Fig. 2 and table), and their intensity confirmed the proportion of terminal and substituted glucose residues observed by methylation. Rather high number of terminal residues in glucan is probably explained not only by comparatively low molecular mass of the native polysaccharide, but also by its possible partial depolymerization during the processes of methylation and Smith degradation.

After the removal of copper ions from copper complex, polysaccharide preparation was obtained that gave

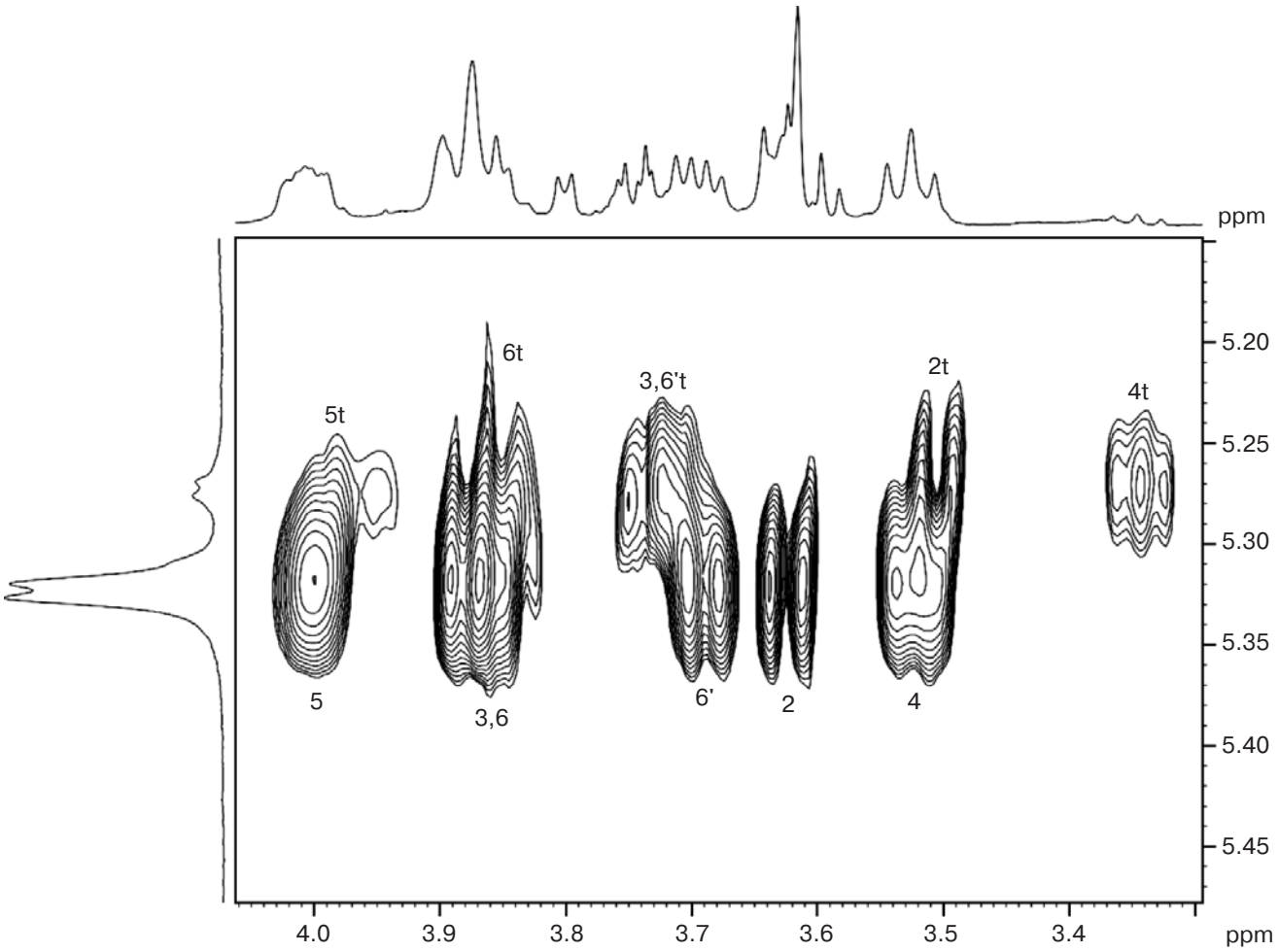


Fig. 2. Part of TOCSY spectrum of (1→3)-α-D-glucopyranan from *G. lucidum*. Proton positions in α-D-glucopyranose residues are numbered, and protons of terminal non-reducing residues are marked with "t".

xylose and mannose during hydrolysis in molar proportion 3 : 4 with a small amount of glucose (xylomannan I). The D-configuration of mannose was determined from chromatographic mobility of acetylated aminodeoxymannitol obtained from the polysaccharide hydrolysate and L-α-methylbenzylamine by the reaction of reductive amination [27]. Unfortunately, we could not get satisfactory chromatographic discrimination of analogous xylose derivatives, though D-configuration of xylose was determined from the ^{13}C-NMR spectral data (see below).

The ^{13}C-NMR spectrum of xylomannan I was complex and could not be easily interpreted. To simplify the structure of polysaccharide I, we used Smith degradation. The resulting modified polysaccharide contained xylose and mannose residues in proportion 1 : 4 (xylomannan II). This result probably meant that xylomannans I and II contain periodate-resistant backbone made of mannose residues. A significant fraction of the xylose residues in xylomannan I have terminal positions and are oxidized by periodate, while the rest of the xylose residues are placed inside the chains and protected from periodate oxidation. Comparative study of xylomannans I and II by NMR spectroscopy confirmed this theory and revealed major structural characteristics of both polysaccharides.

In the ^{13}C-NMR spectrum of initial polymer I (Fig. 3) in the resonance region of anomer carbon atoms, we observed seven broadened and partially overlapping signals of different intensity at δ_C 106.3, 106.0, 103.6, 103.4, 103.1, 102.9, and 102.0. The ^{13}C-NMR spectrum of partially degraded polymer II (Fig. 3) contained in this region four signals at δ_C 105.2, 103.6, 103.4, and 103.1. In the ^{1}H-NMR spectrum of polymer I in the proton resonance region at anomer carbon atoms, we observed broadened signals at δ_H 5.10-5.20, 4.93, and 4.40-4.45. In the ^{1}H-NMR spectrum of polymer II in the resonance regions of anomer protons, we observed the same signals except for the peak at δ_H 4.93.

One-dimensional ^{1}H- and ^{13}C-NMR spectra were interpreted (table) using two-dimensional homonuclear experiments ^{1}H,^{1}H COSY, TOCSY, and ROESY and het-

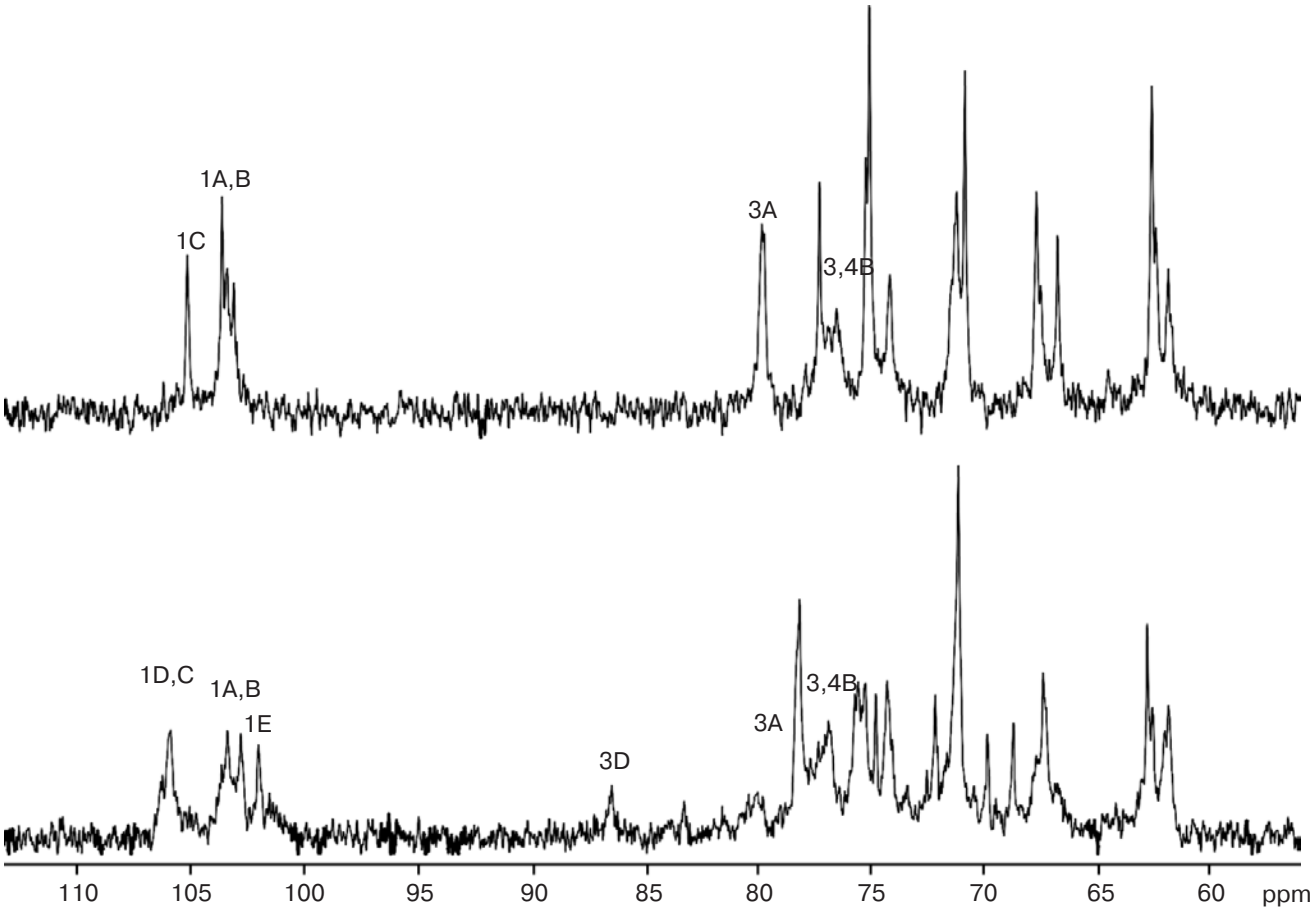


Fig. 3. ^{13}C-NMR spectra of native xylomannan I (bottom) and product of its Smith degradation (xylomannan II; top). Numbers correspond to the carbon atoms in residues A-E (see Scheme and table).

eronuclear experiment ^{1}H,^{13}C HSQC. The analysis of COSY, TOCSY, and ROESY spectra revealed the existence of α-mannopyranose (α-Man*p*) and β-xylopyranose (β-Xyl*p*) residues in polymer II. Identification of signals in the ^{13}C-NMR spectrum of polymer II using analysis of ^{1}H,^{13}C HSQC spectrum and comparing chemical shifts of carbon atoms within residues containing them for corresponding free pyranoses revealed substitution positions in residues by characteristic α-effects of glycosylation. In the spectrum of polymer II, we found signals of 3-substituted (A) and 3,4-disubstituted (B) residues of α-Man*p* and also non-substituted (terminal) residues of β-Xyl*p* (C). Sugar sequence was revealed in analysis of correlation peaks in the ROESY spectrum (Fig. 4). Along with others, correlation peaks for anomer protons of mannose residues A and B and for protons H-3 of such residues were observed in the spectrum (corresponding to (1→3)-link between them). For anomeric protons of xylose C residues, we observed correlation peaks with protons H-4 and H-6,6′ of B residues. Considering 3,4-substitution in B residues, this observation allows us to localize C residues at C-4 of B residues. Thus, polymer II is made of 1→3-linked α-mannopyranose residues, part of which is substituted at C-4 by residues of β-xylopyranose.

Similar NMR study of polysaccharide I showed that besides residues found in polysaccharide II, it contains residues of 3-substituted β-xylopyranose (residue D) and terminal β-mannopyranose (β-Man*p*, residue E). The ROESY spectrum (Fig. 5) particularly contained *trans*-glycoside correlation peak for anomeric protons of residue E and protons H-3 of residue D. So, the chain of initial xylomannan I consists of 1→3-linked α-mannopyranose residues, most of which are substituted at C-4 by residues of β-xylopyranose or disaccharide β-Man*p*-(1→3)-β-Xyl*p*-(1→ (Scheme).

In the ^{13}C-NMR spectra of xylomannans I and II, the signals of C-6 mannose residues carrying xylose residues at C-4 are shifted to higher field by 0.7 ppm (table). According to known expectations about absolute configuration of monosaccharide residues influencing signal position in ^{13}C-NMR spectra of oligo- and polysaccharides [34], such distant effect of glycosylation is observed only in cases where both monosaccharides have

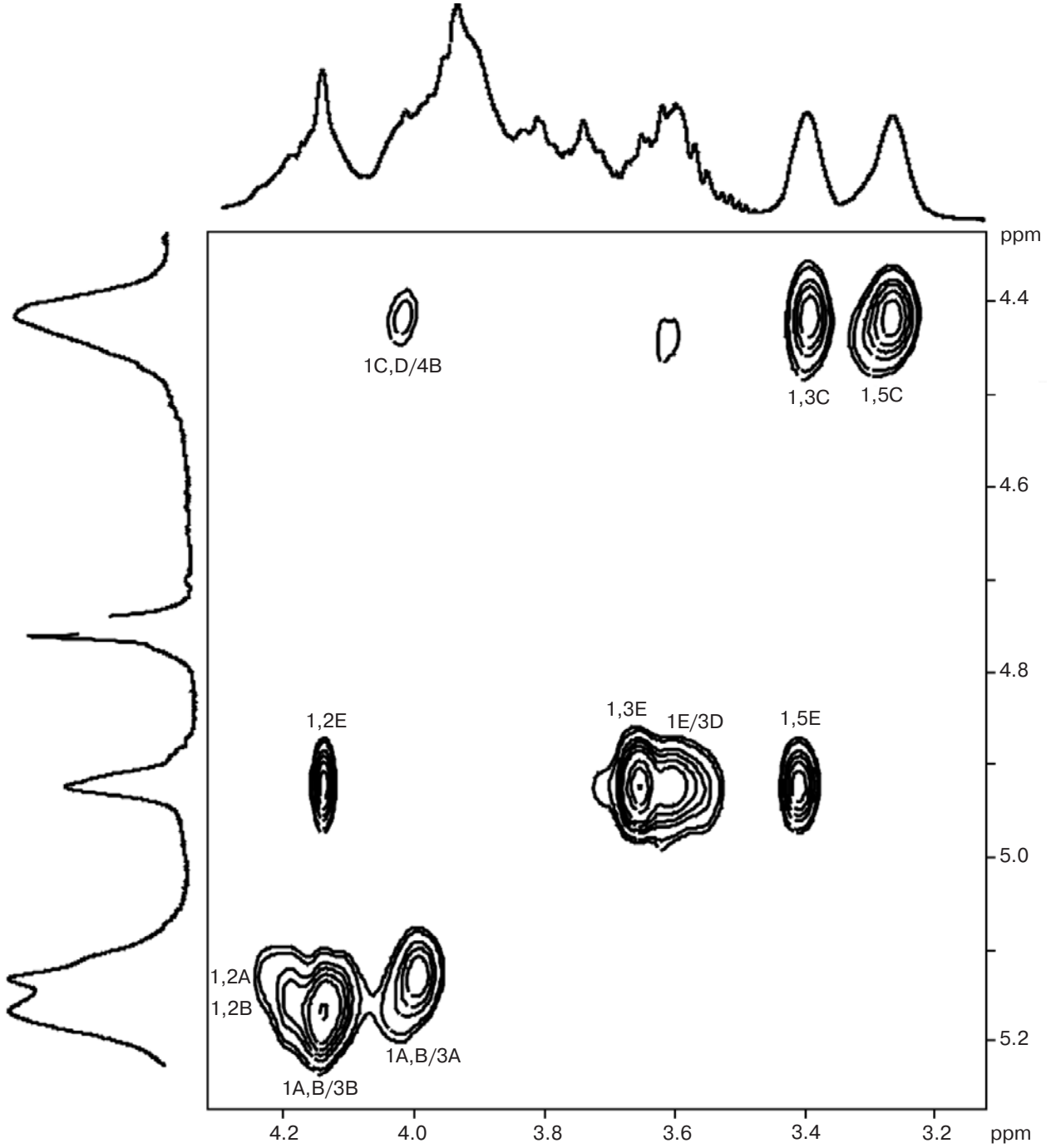


Fig. 4. Part of the ROESY spectrum of xylomannan I. Numbers correspond to the protons in residues A-E (see Scheme and table). For designation of proton correlation signals within residues we use commas, and for spatial contacts through glycoside bonds we use "/".

the same absolute configuration. As D-configuration of mannose residues in xylomannans is proved by an independent method, the spectral data reveals D-configuration of xylose residues incorporated in these polysaccharides.

The structures of the xylomannans proposed based on the spectral data were proved by methylation. In the methylation products of xylomannan II, we found 2,3,4-tri-O-methylxylose, a respective amount of 2,6-di-O-methylmannose, and, as a main component, 2,4,6-tri-O-methylmannose formed from linear regions of the polysaccharide backbone. In the methylation products of xylomannan I we found derivatives corresponding to terminal residues of xylose and mannose, to 3-linked xylose

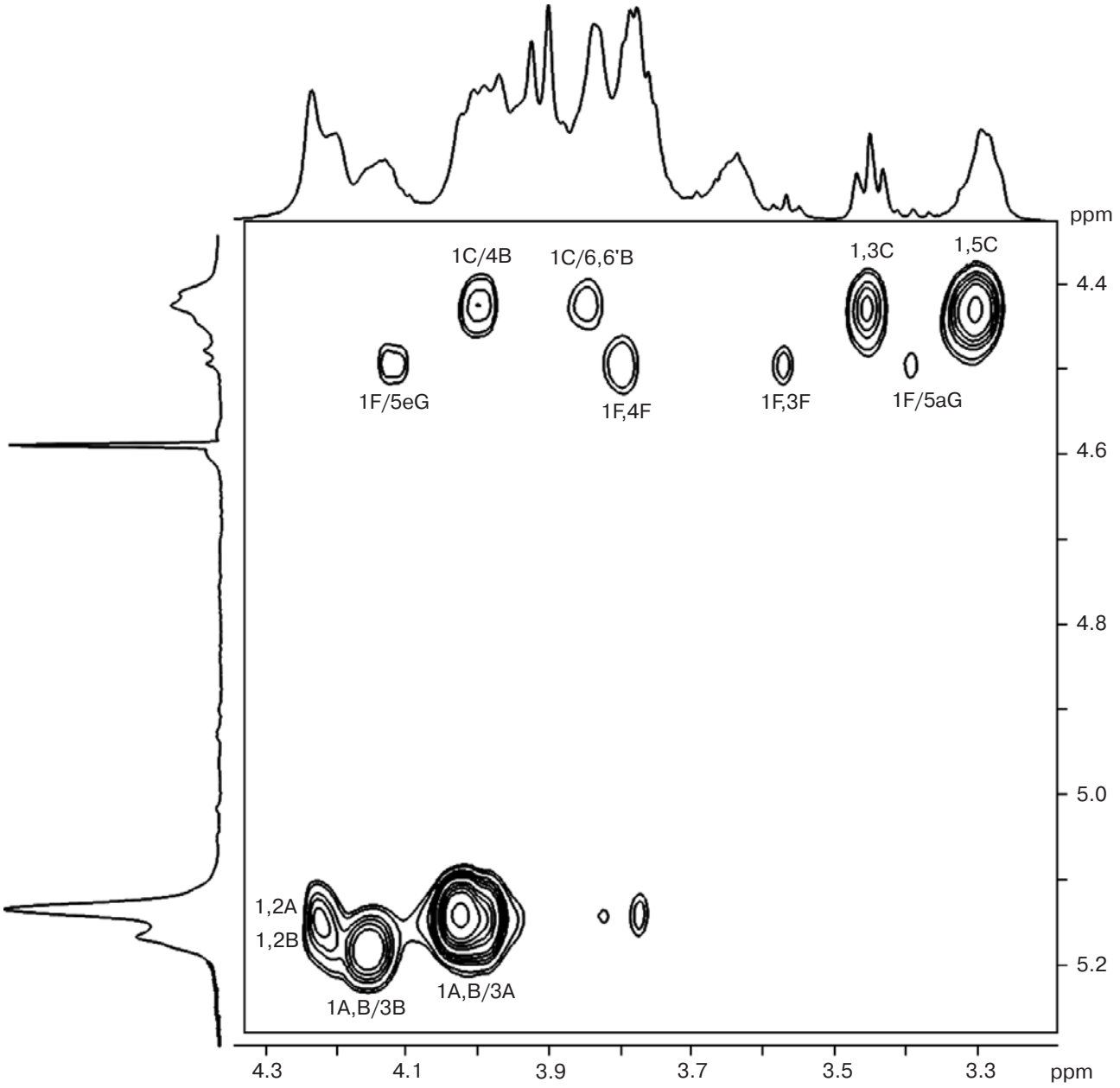
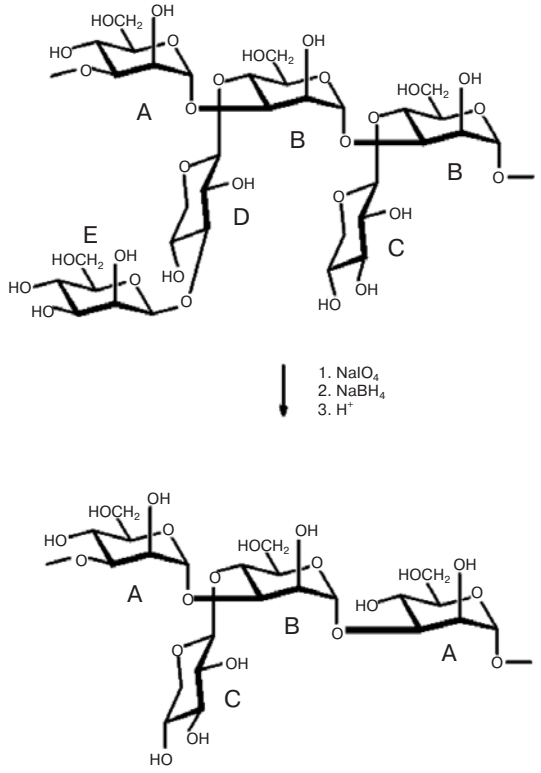


Fig. 5. Part of the ROESY spectrum of xylomannan II (designations are the same as in Fig. 4). Residues of hypothetical disaccharide side branch β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→ are designated as F-(1→4)-G-(1→, 5e and 5a designating equatorial and axial protons, respectively, at C-5 of the G residue.

and mannose residues, and to 3,4-disubstituted mannose residues. As the number of 3-linked xylose residues did not exceed the number of terminal mannose residues, we conclude that these substituted xylose residues are incorporated in disaccharide side chains β-D-Man*p*-(1→3)-β-D-Xyl*p*-(1→ and are therefore protected during periodate oxidation of the polysaccharide. The rest of the xylose residues are present as single branches of the backbone and are destroyed together with β-mannose residues during periodate oxidation. The Scheme only reflects the main structural elements of xylomannans I and II. Molecules of both polysaccharides may have slight differences from the proposed structures. Thus, in the ROESY spectrum of xylomannan II there is a set of low intensity signals that belong to terminal xylose residues (F) that are connected to C-4 residues of xylose (G; Fig. 5). The presence of 4-linked xylose residues is proved by the derivative of 2,3-dimethylxylose (10% of the amount of 2,4-dimethylxylose) that is observed in the methylation products of xylomannan II. This probably means that there are



Scheme for obtaining xylomannan II by Smith degradation of xylomannan I

branches of disaccharide fragments β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→ besides singular side branches from the backbone in polysaccharide molecules. As these fragments were preserved after periodate oxidation, it is obvious that branches in the molecules of native xylomannan I are presented not only by disaccharides β-D-Man*p*-(1→3)-β-D-Xyl*p*-(1→ and that some part of them should have more complex structure.

Thus, our study made during the extraction of *G. lucidum* mycelium, grown by submerged cultivation, revealed water-soluble and alkali-soluble polysaccharides. By fractioning the latter using Fehling reagent, we isolated linear (1→3)-α-D-glucan and highly branched xylomannan containing backbone made of (1→3)-linked α-D-mannopyranose residues, most of which was substituted at position 4 by single residues of β-D-xylopyranose and disaccharide residues of β-D-Man*p*-(1→3)-β-D-Xyl*p*-(1→.

Linear (1→3)-α-D-glucans were previously extracted from many fungi [35] including *G. lucidum* [11, 12]. It is known that they have comparatively low antitumor activity, but this activity can be significantly intensified by chemical modification of polysaccharide molecules, for example, by sulfation or carboxymethylation [12, 36-38].

Xylomannans, like the ones isolated in this work, are less widespread and are not so much studied. Polysaccharide, containing (1→3)-α-D-mannan backbone, partially substituted at position 4 by β-D-xylopyranose residues or small chains of (1→4)-linked β-D-xylopyranose residues, was isolated (also using precipitation of copper complex) from alkali extract of cell walls of the fungus *Polyporus tumulosus* [39]. Xylomannan, similar in structure but containing α-D-xylopyranose terminal residues, was found in the fungus *Armillaria mellea* (it is interesting to note that this polysaccharide was not precipitated by Fehling reagent) [40]. The most similar to the described in our work xylomannan from the fungus *Flammulina velutipes* has the same backbone and branches represented by groups β-Xyl*p*-(1→3)-β-Xyl*p*-(1→, linked at position 4 of the backbone, thought it does not contain terminal residues of β-D-mannopyranose [41]. These residues were recently discovered in molecules of a more complex polysaccharide, glucuronoxylomannan from the fungus *Tremella mesenterica* [42]. Spectral data presented in the cited article confirms the signal identification in NMR spectra made in our work (some differences in positions of single signals are explained by different conditions of spectrum registration and different position of formally same monosaccharide residues in molecules of different polysaccharides). The presence of two types of D-mannopyranose residues in the xylomannan I molecule, α-mannose in the backbone and β-mannose in terminal positions of branches, is an interesting and quite rare feature of its structure. Studying biological activity of this unusual polysaccharide, as well as a product of its chemical modification (xylomannan II), will be the subject of our further research.

REFERENCES

1. Wasser, S. P. (2002) *Appl. Microbiol. Biotechnol.*, **60**, 258-274.
2. Borchers, A. T., Keen, C. L., and Gershwin, M. E. (2004) *Exp. Biol. Med. (Maywood)*, **229**, 393-406.
3. Zhang, M., Cui, S. W., Cheung, P. C. K., and Wang, Q. (2007) *Trends Food Sci. Technol.*, **18**, 4-19.
4. Stone, B. A., and Clarke, A. E. (1992) *Chemistry and Biology of (1→3)-β-Glucans*, LaTrobe University Press, La Trobe University, Victoria, Australia.
5. Zekovic, D. B., Kwiatkowski, S., Vrvic, M. M., Jakovljevic, D., and Moran, C. A. (2005) *Crit. Rev. Biotechnol.*, **25**, 205-230.
6. Lull, C., Wichers, H. J., and Savelkoul, H. E. J. (2005) *Mediators Inflammation*, **2**, 63-80.
7. Descroix, K., Ferrieres, V., Jamois, F., Yvin, J. C., and Plusquellec, D. (2006) *Mini Rev. Med. Chem.*, **6**, 1341-1349.
8. Lin, Z., and Zhang, H. (2004) *Acta Pharmacol. Sin.*, **25**, 1387-1395.
9. Huie, C. W., and Di, X. (2004) *J. Chromatogr. B*, **812**, 241-257.
10. Paterson, R. R. M. (2006) *Phytochemistry*, **67**, 1985-2001.
11. Chen, J., Zhou, J., Zhang, L., Nakamura, Y., and Norisuye, T. (1998) *Polymer J.*, **10**, 838-843.

12. Bao, X., Duan, J., Fang, X., and Fang, J. (2001) *Carbohydr. Res.*, **336**, 127-140.
13. Bao, X., Liu, C., Fang, J., and Li, X. (2001) *Carbohydr. Res.*, **332**, 67-74.
14. Bao, X.-F., Wang, X.-S., Dong, Q., Fang, J.-N., and Li, X.-Y. (2002) *Phytochemistry*, **59**, 175-181.
15. Bao, X.-F., Zhen, Y., Ruan, L., and Fang, J.-N. (2002) *Chem. Pharm. Bull.*, **50**, 623-629.
16. Miyazaki, T., and Nishijima, M. (1981) *Chem. Pharm. Bull.*, **29**, 3611-3616.
17. Saito, K., Nishijima, M., and Miyazaki, T. (1989) *Chem. Pharm. Bull.*, **37**, 3134-3136.
18. Miyazaki, T., and Nishijima, M. (1982) *Carbohydr. Res.*, **109**, 290-294.
19. Li, Y.-Q., Fang, L., and Zhang, K.-C. (2007) *Carbohydr. Polym.*, **68**, 323-328.
20. Wang, Y.-Y., Khoo, K.-H., Chen, S.-T., Lin, C.-C., Wong, C.-H., and Lin, C.-H. (2002) *Bioorg. Med. Chem.*, **10**, 1057-1062.
21. Ye, L. B., Zhang, J. S., Ye, X. J., Tang, Q. J., Liu, Y. F., Gong, C. Y., Du, X. J., and Pan, Y. J. (2008) *Carbohydr. Res.*, **343**, 746-752.
22. Szedlay, G. (2002) *Acta Microbiol. Immunol. Hung.*, **49**, 235-243.
23. Avtonomova, A. V., Krasnopol'skaya, L. M., and Maksimov, V. N. (2006) *Mikrobiologiya*, **75**, 186-192.
24. Krasnopol'skaya, L. M., Belitsky, I. V., Avtonomova, A. V., Soboleva, N. U., Usov, A. I., Isakova, E. B., Libenzon, A. V., and Bukhman, V. M. (2005) *Uspekhi Med. Mikol.*, **5**, 192-195.
25. Bjorndal, H., Hellerqvist, C. G., Lindberg, B., and Svensson, S. (1970) *Angew. Chem. Int. Ed. Engl.*, **9**, 610-619.
26. Sloneker, J. (1975) *Methods of Carbohydrate Research* (Chorlin, A. Y., ed.) [Russian translation], Mir, Moscow, pp. 22-25.
27. Cases, M. R., Cerezo, A. S., and Stortz, C. A. (1995) *Carbohydr. Res.*, **269**, 333-341.
28. Bilan, M. I., Grachev, A. A., Ustuzhanina, N. E., Shashkov, A. S., Nifantiev, N. E., and Usov, A. I. (2002) *Carbohydr. Res.*, **337**, 719-730.
29. Mizuno, T. (1999) *Int. J. Med. Mushrooms*, **1**, 9-29.
30. Whyte, J. N. C., and Southcott, B. A. (1970) *Phytochemistry*, **9**, 1159-1161.
31. Jones, J. K. N., and Studley, R. J. (1967) *Methods of Carbohydrate Chemistry* (Kochetkov, N. K., ed.) [Russian translation], Mir, Moscow, pp. 286-288.
32. Usov, A. I., Blanko, F. F., Ivanova, V. S., Bedrina, E. N., Firsova, C. A., Sedakova, L. A., and Funtikova, N. S. (1991) *Bioorg. Khim.*, **17**, 121-125.
33. Goldstein, I. J., Hay, G. V., Lewis, B. A., and Smith, F. (1967) *Methods of Carbohydrate Chemistry* (Kochetkov, N. K., ed.) [Russian translation], Mir, Moscow, pp. 471-478.
34. Lipkind, G. M., Shashkov, A. S., Knirel, Y. A., Vinogradov, E. V., and Kochetkov, N. K. (1988) *Carbohydr. Res.*, **175**, 59-75.
35. Gorin, P. A. G., and Barreto-Bergter, E. (1983) *The Polysaccharides* (Aspinall, G. O., ed.) Vol. 2, Academic Press, N. Y., pp. 365-409.
36. Kiho, T., Yoshida, I., Nagai, K., Ukai, S., and Hara, C. (1989) *Carbohydr. Res.*, **189**, 273-279.
37. Zhang, L., Zhang, M., Zhou, Q., Chen, J., and Zeng, F. (2000) *Biosci. Biotechnol. Biochem.*, **64**, 2172-2178.
38. Huang, Q., Zhang, L., Cheung, P. C. K., and Tan, X. (2006) *Carbohydr. Polym.*, **64**, 337-344.
39. Angyal, S. J., Bender, V. J., and Ralph, B. J. (1974) *Biochim. Biophys. Acta*, **362**, 175-187.
40. Bouveng, H. O., Fraser, R. N., and Lindberg, B. (1967) *Carbohydr. Res.*, **4**, 20-31.
41. Smiderle, F. R., Carbonero, E. R., Mellinger, C. G., Sassaki, G. L., Gorin, P. A. J., and Iacomini, M. (2006) *Phytochemistry*, **67**, 2189-2196.
42. Vinogradov, E., Petersen, B. O., Duus, J. O., and Wasser, S. (2004) *Carbohydr. Res.*, **339**, 1483-1489.