The American Journal of Applied Sciences
(ISSN – 2689-0992)
Published: January 19, 2021 | **Pages:** 9-17
Doi: https://doi.org/10.37547/tajas/Volume03Issue01-03

IMPACT FACTOR
2020: 5. 276
OCLC - 1121105553

Research On The Composition And Structure Of B -Glucans Isolated From Basidiomycete Raw Materials Inonotus Hispidus

Gulnoza Abduvakhobovna Khalilova
Basic Doctoral Student, A.S. Sadykov Institute Of Bioorganic Chemistry Of The Academy Of Sciences Of Uzbekistan

Abbaskhan Sabirkhanovich Turaev
Doctor Of Chemistry, Academician, Chief Scientific Researcher, A.S. Sadykov Institute Of Bioorganic Chemistry Of The Academy Of Sciences Of Uzbekistan

Bahtiyor Ikromovich Muhitdinov
Doctor Of Philosophy In Chemical Sciences, Senior Scientific Researcher, A.S. Sadykov Institute Of Bioorganic Chemistry Of The Academy Of Sciences Of Uzbekistan

Albina Vasilevna Filatova
Doctor Of Technical Sciences, Leading Researcher, A.S. Sadykov Institute Of Bioorganic Chemistry Of The Academy Of Sciences Of Uzbekistan

Saidakhon Bokijonovna Haytmetova
Candidate Of Technical Sciences, Senior Research Fellow, A.S. Sadykov Institute Of Bioorganic Chemistry Of The Academy Of Sciences Of Uzbekistan

Nodirali Sokhobatalievich Normakhamatov
Doctor Of Chemical Sciences, Senior Research Fellow, A.S. Sadykov Institute Of Bioorganic Chemistry Of The Academy Of Sciences Of Uzbekistan

ABSTRACT

This article highlights the conducted researches on the composition and structure of β-glucans isolated from the basidiomycete raw material Inonotus hispidus. By means of using the alditol acetate method, as well as by UV and IR methods, one-dimensional (^{13}C NMR, ^{1}H NMR), two-dimensional (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC) NMR spectroscopy, the composition and molecular structure of polysaccharides were determined and their branching was proved. It was clarified that the composition of the polysaccharide fractions consists mainly of glucose residues (68-100%), as well as residues of fructose, xylose, mannose and galactose as minor monosaccharides. NMR spectroscopic studies of the specimens showed that the obtained polysaccharides consist of branched glucan structures linked by α- and β-glycosidic bonds. In the structure of β-glucans, the main chain is linked mainly through β-1,3-, partially β-1,4-glycosidic bonds, the branched parts consist of one or more β-D-glucose residues that are linked β-1,3- glycosidic bonds, as well as with the main chain, mainly through α- or β-1,6-glycosidic bonds. The results of the study of molecular parameters showed that the MW of β-glucans of basidiomycetes are in the range of 9100-9900 Da, MWD - 1.2-1.5.

KEYWORDS

β-glucans, basidiomycetes, polysaccharides, branched structure

INTRODUCTION

Currently, one of the most promising scientific directions in the world is the study and isolation of new biologically active substances. Basidiomycetes, which are sources of a number of biologically active substances, including β-glucans [1-4], are the priority group for the study. In fungi of various genera (Agaricus, Boletus, Paxillus, Pleurotus), polysaccharides were found in which glucose monomers are linked by a beta-glycosidic bond. By their nature, these polysaccharides are water-soluble β-glucans with a branched structure.

One of the most common forms of polysaccharides, glucose polymers are Beta-D-glucans, linked by a 1→3 linear beta-glycosidic chain and differ from each other in length and branched structure. Branches of beta-D-glucans are species-specific, for example, the beta-D-glucans of the fungus have 1→6 lateral branches, while the beta-D-glucans of bacteria have 1→4 lateral branches [5]. β-Glucans, which are isolated from different sources,vary greatly in their structure and composition. For instance, macromolecules of β-glucans of cereals have a linear structure and contain blocks of β-D-glucopyranose residues linked by β-(1→4)-glycosidic bonds. Molecules of β-glucans of yeast and fungi have a branched structure and consist of β-D-glucopyranose residues linked by β-(1→3) glycosidic bonds, and the side branches are connected to the main chain at O-6 positions [6]. Depending on the structure of the molecule, its effectiveness also varies, so the results of studies with one type of beta-1,3/1,6-glucans cannot be automatically transferred to another one.

β-Glucans are used in cosmetology, medicine, and the food industry. The most important physiological effect of β-glucans is their ability to increase the activity of the human immune system [7-10].

Analysis of literature data shows that basidiomycete raw materials are promising raw materials for the production of β-glucans.

In this regard, the aim of this work is to study the composition and structure of β-glucans isolated from the basidiomycete raw material *Inonotushispidus*.

EXPERIMENTAL PART

The objects of research were the fruit bodies of the basidiomycetes *Inonotushispidus* (locally growing mushroom). Basidiomycetes were collected from areas of natural growth in the Tashkent region and the Fergana Valley. The isolation of water-soluble polysaccharides was carried out by water extraction methods [11, 12].

Ion exchange chromatography. A specimen of a water-soluble polysaccharide (100 mg) was dissolved in 5 ml of distilled water and poured to a column (14×3 cm) with DEAE-52 (Sigma-Aldrich Chemie GmbH, Germany) cellulose. Elution of polysaccharides was carried out sequentially with 0-1M gradient NaCl solutions at a rate of 60 ml/h. Fractions of 10 ml were collected. The yield of polysaccharides from the column was monitored by the phenol-sulfuric acid method. Fractions corresponding to individual peaks were pooled, concentrated, dialyzed and lyophilized.

Gel filtration of polysaccharides. Specimens of polysaccharides (50 mg) were dissolved in 2 ml of water and applied to a column (41x2) with Sephadex G-75. The spare volume of the column is 32 ml. The yield of polysaccharides from the column was monitored by the phenol-sulfuric acid method. The fractions were pooled, concentrated to a minimum volume, dialyzed and lyophilized [13].

Determination of the monosaccharide composition of β-glucans

By using the alditol acetate method, the monosaccharide composition of individual polysaccharides was determined. The polysaccharide (5 mg) and 2 M trifluoroacetic acid (3 ml) were placed in an ampoule, hydrolyzed at 110°C for 6 hours. To remove the trifluoroacetic acid hydrolyzate, the hydrolyzate was diluted three times with 5 ml of dry methanol solution. Hydroxylamine hydrochloride (10 mg), isonitol (2 mg) were added to the dry hydrolyzate and dissolved in pyridine (5 ml). The solution was heated at 90°C for 30 minutes, quickly cooled to room temperature, acetic anhydride (0.5 ml) was added and acetylated for 30 minutes at 90°C. The reaction mixture was dried under a stream of nitrogen, which was dissolved in chloroform (0.5 ml) and filtered through a syringe filter (0.45 m).In the way described above, alditol acetate derivatives of monosaccharide standards (D-Glc, D-Gal, D-Rib, D-Ara, L-Rha, D-Man, D-Xyl and D-Fru) were prepared. The synthesized alditol acetate derivatives were analyzed by GC-MS (Thermo Finnigan TRACE 2000/MS column, DB-5MS (30 m×0.25 mm×0.25 mm), temperature program varied from 180 to 270°C at 20°C/min, with holding 270°C for 25 min). The peaks corresponding to alditol acetates and their fragments were determined by their mass spectra and GC separation times. The ratio of monosaccharides to polysaccharides was found out by comparing the peak areas.

The determination of molecular weights. M_w and the distributions of molecular weight were determined by size exclusion liquid chromatography on a liquid chromatograph, consisting of a Merk-HitachiL-6000A plunger pump, a ShodexRI-101 refractometric detector, a DAWNNSP multi-angle laser light scattering detector (MLLS), WattTechnology (USA), a hand injector 2104, an eluent degasser, and two PLAquagel-OHMixed chromatographic columns thermostated at 25°C, connected in series as described in [13].

The molecular weights M_w of the synthesized samples and their molecular weight distribution were also determined by sedimentation on an MOM device (Hungary).

UV-spectroscopy. Spectrophotometric determinations were conducted on a Shimadzu UV-VIS 1280 spectrophotometer (Shimadzu Europa GmbH, Germany).

IR-spectroscopy. IR spectra of the samples under study were recorded on a PerkinElmer 2000 system IR Fourier spectrometer in the frequency range 400-4000 cm^{-1} in a KBr tablet

NMR spectroscopy. ^{13}C NMR, ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz and Bruker Avance 600 MHz spectrometers in D_2O at 50°C, at a frequency of 100 MHz and a pulse width of 30 °, for 0.3 seconds and with a relaxation delay of 3 seconds.

RESULTS AND THEIR DISCUSSION

In order to isolate polysaccharides, the fruit bodies of basidiomycetes, (locally growing mushroom) *Inonotushispidus*were used, which were dried to constant weight in an DC-80-01 SPU drying cabinet at a temperature of 40-50°C.

A polysaccharide complex (polysaccharides and melanin) has been isolated by water extraction methods from basidiomycete raw materials.

The fraction was separated into polysaccharides and melanin and further purified by deproteinization (purification from proteins and peptides), ion exchange, and gel chromatography [14,15]. A sample of polysaccharides, purified and separated from melanin, was analyzed.

When analyzing the polysaccharide sample by the method Savage using UV spectroscopy, no peaks corresponding to proteins at 280 and 260 nm were observed. While passing a sample of polysaccharides through DEAE cellulose, it was first washed in distilled water and then NaCl was added. Over time, the concentration of the NaCl solution increased to 1.0 M. The neutral polysaccharides were separated by washing with distilled water, which gave one peak. When the sample was washed with NaCl, the anionic polysaccharides were not separated. This indicates that this sample is composed of neutral polysaccharides.

Gel chromatography was performed on Sephadex G-75, in order to determine the homogeneity of the fraction of the original polysaccharide, as well as to purify it from accompanying impurities, in order to further study the composition and structure of carbohydrate chains. During the separation process, it was determined that the polysaccharide sample consisted of homogeneous polysaccharides (according to Figure 1). The content of carbohydrates in the purified sample was tested by the phenol-sulfuric acid method and amounted to 95 - 99.4%.

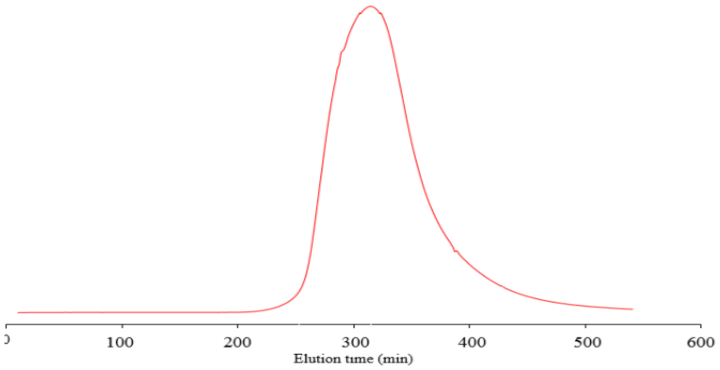

Figure 1. Gel chromatogram of the polysaccharide fraction

The molecular weight characteristics of the obtained fraction of polysaccharides were determined using the gel filtration method.

The research on the polysaccharides isolated from the basidiomycete raw material Inonotus hispidus showed that molecular weight features are characterized by a relatively low polydispersity value, which indicates that the polysaccharides are homogeneous. The results show that the molecular weight of the polysaccharides of the aqueous GB fraction was 9100-9900 Da, the MWD was from 1.4 to 2.1.

Further studies were aimed at determining the monosaccharide composition of the obtained sample. Using GC-MS of the alditol acetate method, the monosaccharide composition of polysaccharides isolated from Inonotus hispidus by aqueous and alkaline extractions was established. From the results obtained, it was found that polysaccharides consist mainly of glucose residues (in accordance with Figure 2). It has been established that the BG polysaccharide consists of glucose residues, and other monosaccharides are found in their composition in trace amounts, as a result of which they are not included in the basic structure.

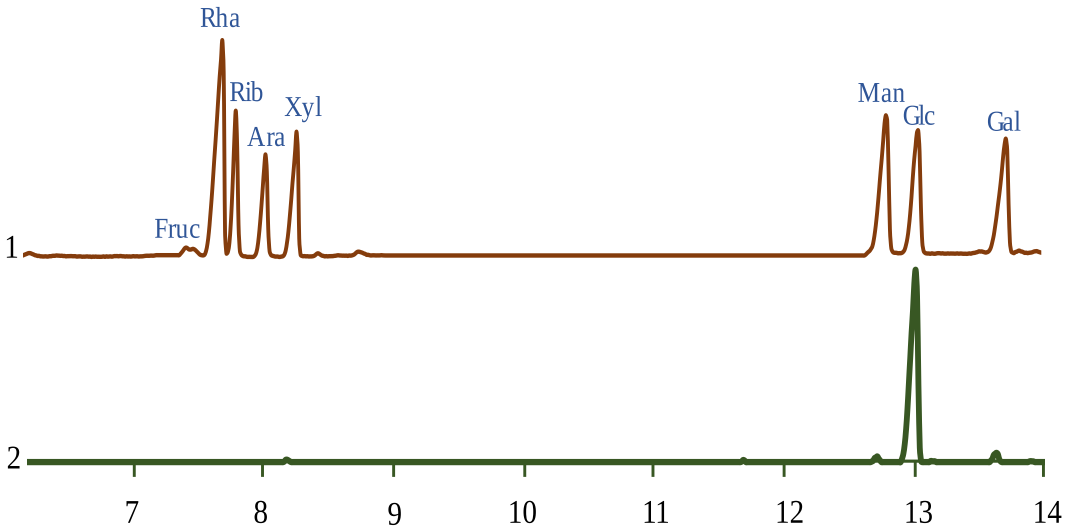

Figure 2. GC-MS spectra of alditol acetates derivatives of standard monosaccharides, as well as monosaccharides in the isolated polysaccharide BG

The results of the study showed that the composition of the BG polysaccharide sample may contain residues of fructose, xylose, mannose and galactose as minor monosaccharides. Table 1 shows the monosaccharide composition of the BG aqueous fraction sample.

Table 1. Monosaccharide composition of the sample of the aqueous fraction of BG polysaccharides

Specimen	***Monosaccharide composition***, %							
	Fruc	Rha	Rib	Ara	Xyl	Man	Glc	Gal
BG	2,70	-	-	-	2,70	18,92	67,57	8,11

As can be seen from the table, in the composition of the BG polysaccharide, in addition to glucose residues, there are also residues of mannose (18.92%), xylose, galactose and fructose in significant amounts.

IR spectroscopic studies of the BG sample were carried out.All absorption bands characteristic of beta-glucans were found in the IR spectra of the polysaccharide sample under study. The absorption band in the range of 2850-2900 cm^{-1} corresponds to stretching vibrations of the carbon atom of C-H bonds of C6 polysaccharides.

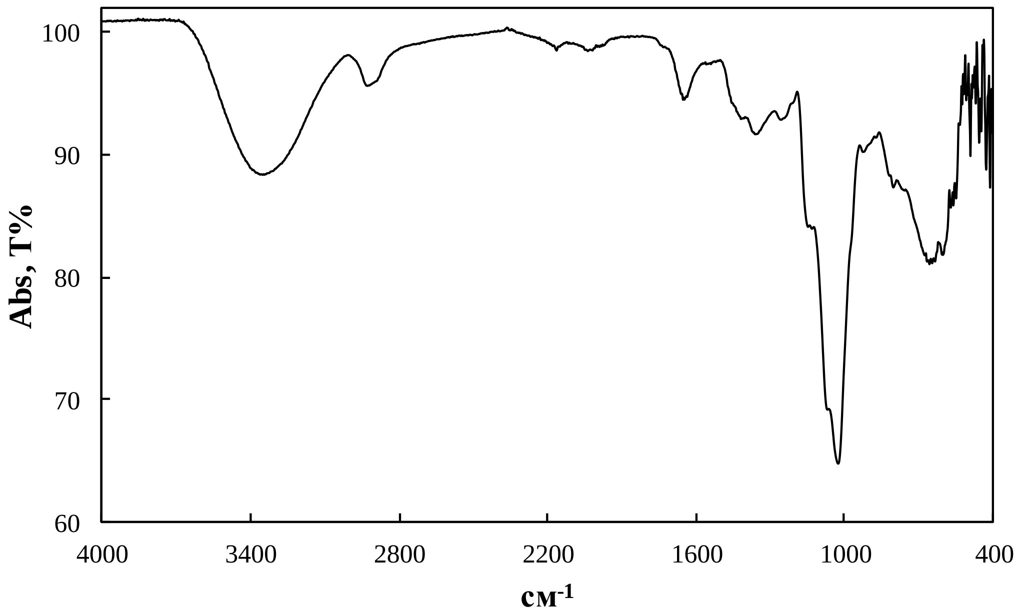

Figure 3. IR spectrum of the obtained polysaccharides from the aqueous fraction BG

In the IR spectra of the obtained sample, the absorption bands corresponding to polysaccharides were observed at 3430 cm^{-1}, 2920 cm^{-1}, 1630 cm^{-1}, 1420-1380 cm^{-1}, 1200-750 cm^{-1}. Also, absorption bands of stretching vibrations of O-H and C-H were observed in the regions of 3400 cm^{-1} and 2900 cm^{-1}, respectively. In the regions of 1630 cm^{-1} and 1420-1380 cm^{-1}, absorption bands corresponding to total polysaccharides were observed [16]. In the region 1200-950 cm^{-1}, absorption bands of stretching vibrations corresponding to C-O and C-C bonds were observed. In the range 950-750 cm^{-1}, absorption bands were observed corresponding toC1-H bonds between the first anomeric carbon atom and hydrogen (in accordance with Figure 3)

Further study of a sample of polysaccharides (β-glucans) BG was performed using one-dimensional (^{13}C NMR, ^{1}H NMR), two-dimensional (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC) NMR spectroscopy (in accordance with Figure 4). In the ^{13}C NMR spectra of the samples, the corresponding signals were observed in the range of 105-60 ppm. The corresponding peaks were also observed for α-anomers of the C-1 carbon atom of the glucopyranose ring of polysaccharides in the range 99-101 ppm, for β-anomers in the range between 102 and 105 ppm. Peaks corresponding to C-2, C-3, C-5 and C-5'(formed by C-6 glycosidic binding) were found in the 71-75 ppm range.

Peaks were also observed in the 69-70 ppm range, corresponding to carbon atoms C-4 and C-4 linked by a glycosidic bond. The peaks corresponding to the C-4 carbon atom linked by a glycosidic bond formed in the range 79-85 ppm. In the range 66-70 ppm peaks were observed corresponding to the C-6 carbon atom involved in glucoside binding. The peaks corresponding to the carbon atom C6 were observed in the range 60-64 ppm. In the ^{1}H NMR spectra of the samples, peaks in the range of 4.8-5.4 ppmcorrespond to the anomeric hydrogen atoms H-1 in α-D-glucans. The peaks corresponding to the carbon atom C6 were observed in the range 60-64 ppm. In the ^{1}H NMR spectra of the samples, the peaks in the range 4.5-4.8 ppmcorrespond to the anomeric hydrogen atoms H-1 in β-D-glucans. In the range of 3.2-4.0 ppm ^{1}H-NMR spectrum, peaks corresponding to hydrogen atoms H-2, H-3, H-4, H-5, H-6 were observed (Table 2).

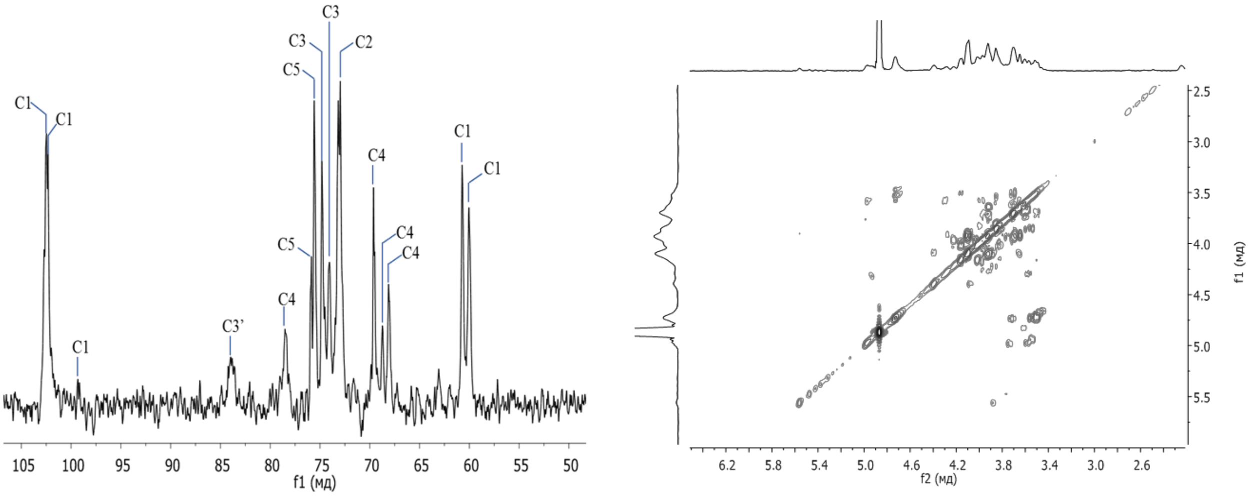

Figure 4.^{13}C NMR, and ^{1}H-^{1}H COSY, spectra of polysaccharides isolated from the aqueous fraction (BG)

Table 2. ^{1}H-^{13}C NMR signals of polysaccharide samples isolated from the aqueous fraction (BG)

Glycosidic bond	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
1,3-Glcp	102,3/4,57	73,1/3,47	84,1/3,80	69,6/3,48	75,9/3,49	60,7/3,72, 3,92
1,3,6-Glcp	102,5/4,56	72,9/3,42	84,1/3,76	69,5/3,48	75,9/3,72	68,1/3,94, 4,35
1,4-Glcp	102,5/4,23	72,9/3,38	74,1/3,69	78,5/3,72	75,6/3,72	60,1/4,04, 4,10
1,4,6-Glcp	102,5/4,22	72,9/3,42	74,1/3,54	78,5/3,49	75,9/3,62	68,7/3,85, 4,18
Terminal-Glcp	102,5/4,01	73,1/3,33	74,1/3,52	70/3,40	75,93,48/	60,7/3,71, 3,87
1,6-Glcp	102,5/3,90	73,1/3,35	74,1/3,52	69,6/3,50	75,9/3,62	68,7/3,85, 4,18

NMR spectroscopic studies of a sample of polysaccharides showed that the obtained polysaccharides consist of glucan structures linked by α- and β-glycosidic bonds. The results show that in the structure of β-glucans, the main chain is linked mainly through β-1,3-, partially β-1,4-glycosidic bonds. The branched parts consist of one or more β-D-glucose residues, which are linked by β-1,3-glycosidic bonds, and also to the main chain, predominantly through an α- or β-1,6-glycosidic bond. Below is the general structure of a polysaccharide sample isolated from *Inonotushispidus.*

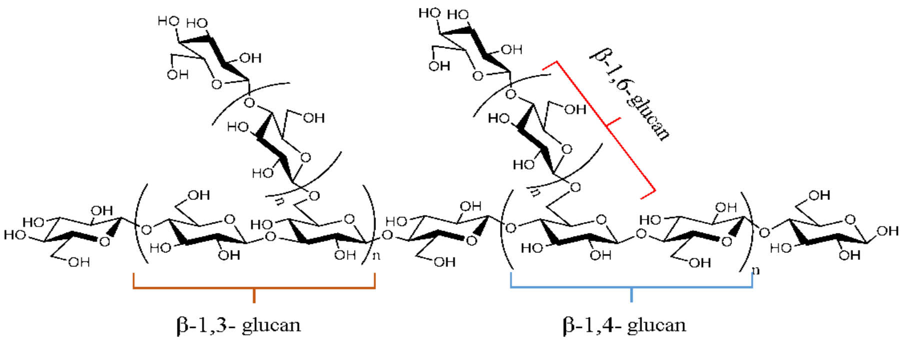

Figure 5. Structure of β-glucans isolated from Inonotushispidus

Structural studies have established that the obtained polysaccharides consist of β-1,3/6-branched glucan structures, having mainly β-1,4 / 1,3-glycosidic bonds and branched with one or more β-1,6-glycosidic bonds.

CONCLUSIONS

are as follows:

- The physicochemical properties of the samples obtained by IR and UV spectroscopy were studied. The composition and molecular structure of the obtained polysaccharide samples have been established by gas chromatography, IR, one-dimensional and two-dimensional NMR spectroscopy;
- It was found that the composition of the polysaccharide fractions consists mainly of glucose residues (68-100%), as well as minor monosaccharides up to 32% residues of fructose, xylose, mannose and galactose.
- Structural studies determined that the obtained polysaccharides consist of β-1,3/6-branched glucan structures, having mainly β-1,4 / 1,3-glycosidic bonds and branched with one or more β-1,6-glycosidic bonds.

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