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POLYSACCHARIDES OF SAPONIN-BEARING PLANTS. V. STRUCTURAL INVESTIGATION OF GLUCANS A, B, AND C AND THEIR OLIGOSACCHARIDES FROM *Biebersteinia multifida* PLANTS

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The partial acid hydrolysis of glucans A, B, and C has given five linear oligosaccharides consisting of α-(1→6)-bound glucopyranose residues, and their structures have been investigated by reduction, peroxidate oxidation, methylation, mass spectrometry, and ^{13}C NMR spectrometry. Chemical structures are proposed for glucans A, B, and C.

In preceding papers [1] we have shown that the main chains of glucans A, B, and C from *Biebersteinia multifida* consist of α-(1→6)-bound glucopyranose residues having branching at the C-3 atom of a glucopyranose, and that these compounds are weakly-branched glucans.

In the present paper we consider the structure of the oligosaccharides (OSs) obtained on the partial hydrolysis of glucans A, B, and C. In the product of the partial hydrolysis of glucan A, with the aid of PC we detected glucose and five oligosaccharides, (**1-5**) (scheme 1). The products of partial hydrolysis were separated preparatively by PC, giving the individual OSs the characteristics of which are shown in Table 1.

The structures of the oligosaccharides were studied by chemical and spectral methods. On the complete acid hydrolysis of oligosaccharides (**1-5**) only D-glucose was detected by PC and GLC, while only glycerol was identified by PC and TLC in the products of their periodate oxidation and Smith degradation. Consequently, in the OSs the glucopyranose residues were linked with one another by 1→6-bonds. These results were confirmed by the methylation of the OSs (Table 1). After complete acid hydrolysis of the methylation products, 2,3,4-tetra-OMe-*D*-Glcp and 2,3,4-tri-OMe-*D*-Glcp were detected. The ratios of tetramethylglucose and trimethylglucose and also those of sorbitol and glucose in the reduction products made it possible to determine the degrees of polymerization of the OSs (Table 1).

TABLE 1. Characteristics of Oligosaccharides (**1-5**) from the Glucans of *B. multifida*

OS	$[\alpha]_D^{21}$, deg (*c* 1.0;water)	Reduction product (hydrolysate)		Methylation product (hydrolysate)		DP
		sorbitol	glucose	2,3,4,6-tetra-OMe-*D*-Glcp	2,3,4-tri-OMe-*D*-Glcp.	
1	+120	1	1	1	1	2
2	+135	1	2	1	2	3
3	+151	1	3	1	3	4
4	+162	1	4	1	4	5
5	+168	1	5	1	5	6

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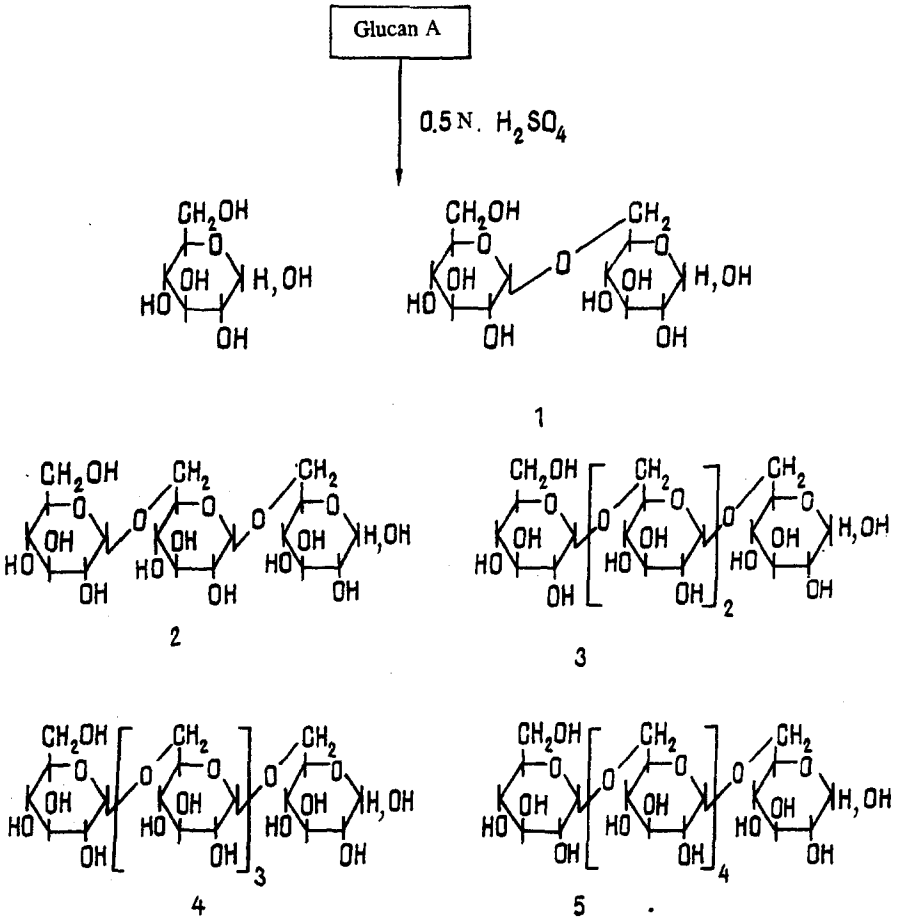
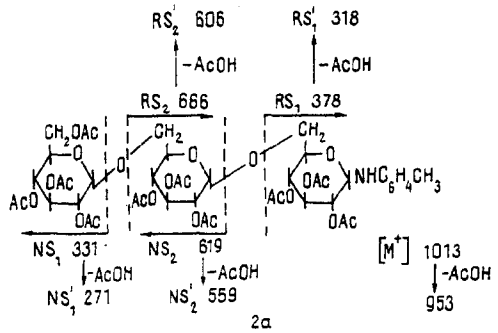


Scheme 1. Partial hydrolysis of glucan A from *B. multifida*.

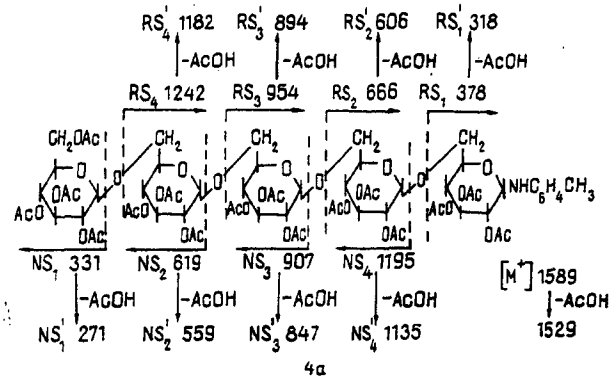
The mass-spectrometric study of oligosaccharides (**2**) and (**4**) was carried out with fully acetylated N-*p*-tolylglycosylamines (schemes 2 and 3). As can be seen from schemes 2 and 3, the mass spectra of OSs **2** and **4** contained peaks corresponding to molecular ions with *m/z* 1013, 1589, which showed their degrees of polymerization as 3 and 5.

The mass spectrum of the N-*p*-tolylglycosylamine derived from the glucotriose (**2a**) had fragments with *m/z* 331 and 619 arising as the result of the successive splitting out of one and two monosaccharide residues from the nonreducing end, and with *m/z* 378 and 666, consisting of one or two monosaccharide units from the reducing end.

In the mass spectrum of (**4a**), in addition to the signals described above, characteristic fragments with *m/z* 907, 954, 1195, and 1242 belonging to the N-*p*-tolylglycosylamine derived from the glucopentose were identified.



Scheme 2. Mass-spectrometric fragmentation of the N-*p*-tolylglycosylamine derived from the glucotriose.



Scheme 3. Mass-spectrometric fragmentation of the N-*p*-tolylglycosylamine derived from the glucopentose.

The mass spectra of (**2a**) and (**4a**) also had the peaks of ions with *m/z* 304, 346, 364, and 377, which are characteristic for 1→6-bound hexoses, the results obtained corresponding to literature information [2].

On the basis of the facts given above, for oligosaccharide (**2**) we propose the structure of O-α-D-glucopyranosyl-(1→6)-O-α-*D*-glucopyranosyl-(1→6)-O-α-*D*-glucopyranose, and for oligosaccharide (**4**) that of O-α-*D*-glucopyranosyl-(1→6)-O-α-*D*-glucopyranosyl-(1→6)-O-α-*D*-glucopyranosyl-(1→6)-O-α-*D*-glucopyranosyl-(1→6)-O-α-*D*-glucopyranose.

Disaccharides, trisaccharides, and tetrasaccharides were also detected in the hydrolysates from the partial hydrolysis of glucans B and C. The isolation, purification, and study of the structures of the oligosaccharides were carried out as in the case of the oligosaccharides from glucan A, and their structures were identical with those of (**1**), (**2**), and (**3**) (scheme 1).

The structures of glucans A, B, and C and their oligosaccharides (**1-5**) were studied by ^{13}C NMR spectroscopy. Below we give the chemical shifts (ppm) of the main strong signals of the carbon atoms of these glucans.

Glucans	C-1	C-2	C-3	C-4	C-5	C-6
A	98.95	72.65	74.65	70.95	71.45	67.00
B	99.00	72.70	74.65	70.95	71.30	67.00
C	99.00	72.70	74.65	71.00	71.50	67.15

As we see, analogous carbon atoms of the glucans were characterized by close chemical shifts that differed only by the intensities of the signals. Signals at 98.95 and 99.00 ppm showed that the glucopyranose residue had the α-configuration. Intense signals at 67.00 and 67.15 ppm corresponded to the C-6 atoms of α-1→6-bound glucopyranose residues.

Figure 1 shows the ^{13}C NMR spectrum of glucan B. The less intense signals in the 61.9 ppm region are characteristic for the C-6 atoms of nonreducing glucopyranoses and those at 67.9 ppm for the C-6 atoms of reducing glucopyranoses. A signal at 82.5 ppm relates to the branched C-3 atom of a glucose residue in the glucan, a signal at 99.8 ppm to C-1 of the same branched glucopyranose residue, and a signal at 93.8 ppm to C-1 of the reducing glucopyranose atom in glucan B [3, 4].

The ^{13}C NMR spectra of oligosaccharides (**1-5**) also had close chemical shifts of the carbon atoms, and the signals differed in intensity as in the case of the glucans:

Oligosaccharides	C-1	C-2	C-3	C-4	C-5	C-6
1	99.00	72.57	74.22	70.72	71.14	66.97
2	98.90	72.57	74.48	70.75	71.38	66.90
3	98.83	72.51	74.48	70.75	71.33	66.84
4	98.83	72.54	74.44	70.70	71.31	66.90
5	98.90	72.59	74.56	70.81	71.38	66.90

As we see, in the spectra of OSs (**1-5**) signals at 98.83-99.00 ppm relate to the C-1 atoms, and those at 66.84-66.97 ppm to the C-6 atoms of α-1→6-bound glucopyranose residues [5].

Figure 2 gives the ^{13}C NMR spectrum of oligosaccharide (**5**), and the same pattern could be observed in the spectra of the OSs (**1-4**). In the spectrum of oligosaccharide (**5**), the less intense signal at 61.74 ppm is characteristic for the C-6 atoms of nonreducing glucopyranose residues, while the signals at 97.25 and 93.36 ppm relate to the reducing anomeric carbon atoms of glucopyranoses having the β- and α-configurations, respectively.

Oligosaccharides (**1-5**) gave no signal at 82.5 ppm, which showed the absence of branching at the C-3 carbon atoms of a glucopyranose residue, i.e., OSs (**1-5**) were linear.

Thus, structures have been proposed for glucans A, B, and C of *B. multifida* on the basis of the results of chemical and spectral methods of investigation.

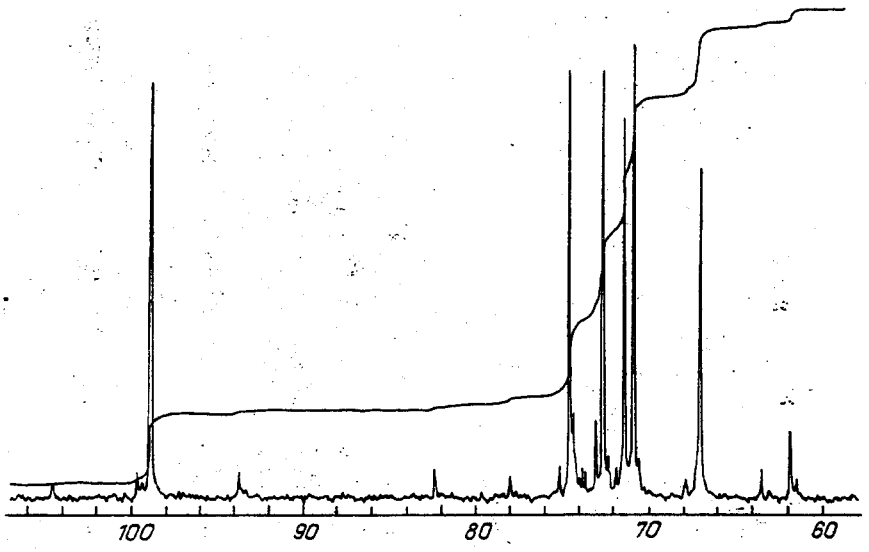


Fig. 1. ^{13}C NMR spectrum of glucan B.

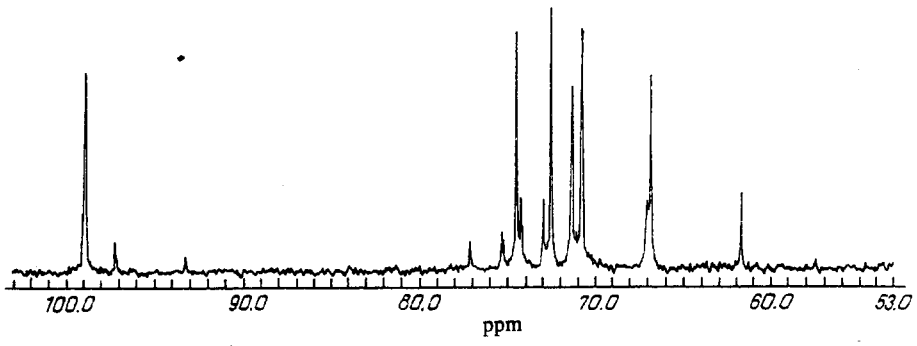
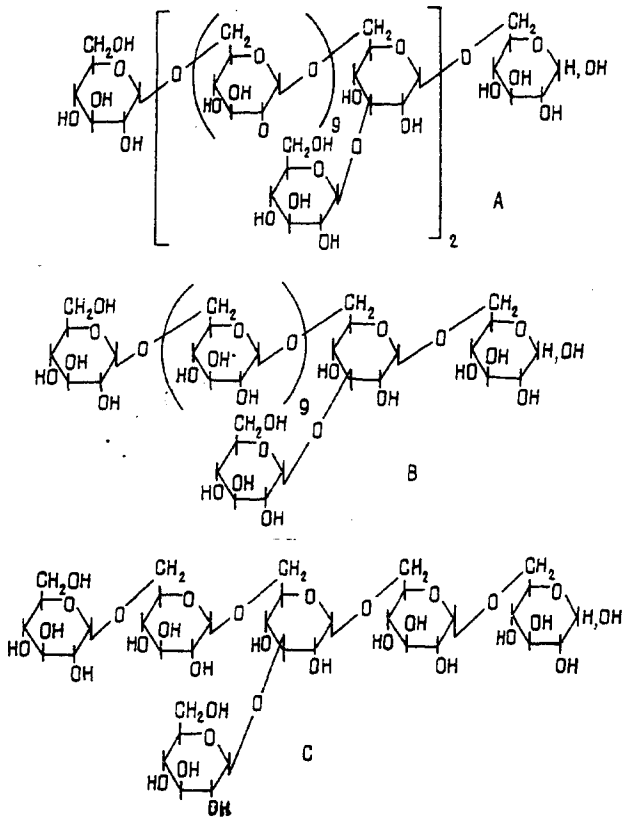


Fig. 2. ^{13}C NMR spectrum of oligosaccharide (**5**).



Structure of glucans A, B, and C of Bieyersteinva multifida

EXPERIMENTAL

TLC was conducted on Silufol UV-254 plates and on type LS 5/40 mm silica gel in the solvent systems: 1) benzene—acetone (1:1), (1:2), and (1:4); 2) chloroform—methanol (9:1); and 3) methyl ethyl ketone—1% NH_4OH (30:1). For PC we used Filtrak FN-3, 11, 16 paper in solvent system 4) butan-1-ol—pyridine—water (6:4:3).

The substances were detected by spraying with the following revealing agents: 1) acid aniline phthalate, and 2) KIO_4—$KMnO_4$—benzidine.

GLC was conducted on a Chrom-5 chromatograph with a flame-ionization detector under the conditions given in [1]. The mass spectra of the individual substances were recorded on a Varian MAT CH-6 instrument with direct injection of the sample into the ion source at a temperature of the ion source of 160-220°C and a temperature of the inlet system of 250-280°C.

The ^{13}C NMR spectra of the poly- and oligosaccharides were taken on a Bruker WR-60 instrument with a working frequency for carbon of 15.08 MHz using complete suppression of spin-spin coupling with protons. Solutions with a concentration of 2-3% in D_2O were prepared and methanol was used as internal standard, its chemical shift relative to TMS being taken as 50.15 ppm.

The specific rotations of the substances were measured on a Zeiss polarimeter in a tube 0.5 dm long with a volume of 1 ml at 20-23°C.

The reduction of the oligosaccharides was achieved by the action of an excess of sodium tetrahydroborate for 5-6 h. The solution was neutralized with KU-2 cation-exchange resin (H^+), filtered, and evaporated to dryness. The dry residue was treated several times with methanol, which was distilled off in vacuum each time. In this way we obtained a reduced product containing no boric acid. The residue has hydrolyzed with 1 N H_2SO_4, neutralized with barium carbonate, treated with KU-2 cation-exchange resin (H^+), and analyzed by PC (system 1, revealing agent 1) and GLC.

Partial Hydrolysis of Glucan A. A solution of 3.5 g of glucan A in 10 ml of 0.5 N H_2SO_4 was heated at 100°C for 1 h. Then it was neutralized with $BaCO_3$, deionized with KU-2 (H^+), and evaporated to a syrup. PC (system 1), revealing agent (1), showed the presence of glucose and of oligosaccharides (**1-5**) with R_f 0.54, 0.28, 0.14, 0.07, and 0.03, respectively, their specific rotations being given in Table 1.

A hydrolysate of glucan A was separated by PC (system 1, revealing agent 1), the zones corresponding to the individual oligosaccharides were cut out and extracted with water, and the extracts were evaporated to dryness. Five chromatographically individual oligosaccharides (**1-5**) were obtained, with yields of 0.38, 0.36, 0.27, 0.21, and 0.23 g, respectively.

In a similar way, glucan B yielded oligosaccharides (**1**), (**2**), and (**3**), and glucan C oligosaccharides (**1**) and (**2**).

Hydrolysis of the Oligosaccharides. Oligosaccharides (**1-5**) (0.01 g each) were hydrolyzed with 0.5 N H_2SO_4 at 100°C for 4 h. After appropriate working up, only glucose was revealed in the hydrolysates by PC (system 1, revealing agent 1).

The methylation and periodate oxidation of the oligosaccharides was carried out as in [1].

Preparation of the Acetylated N-*p*-Tolylglycosylamines. Using the method of Usov and Barbakadze [6], oligosaccharides (**2**) and (**4**) (0.01 g each) yielded oligosaccharides (**2a**) and (**4a**), which were studied by mass spectrometry.

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