POLYSACCHARIDES OF SAPONIN-BEARING PLANTS. IV. STRUCTURE OF GLUCANS A, B, AND C OF *Biebersteinia multifida*

A. O. Arifkhodzhaev and D. A. Rakhimov

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The neutral polysaccharides of the tuberous roots of *Biebersteinia multifida* consist of three weakly branched glucans, A, B, and C. It has been established by periodate and chromic acid oxidation, methylation, and mass spectrometry that in the chains of glucans A, B, and C the monosaccharides are linked to one another by α-1 → 6 bonds and there is branching at the C-3 atoms of the glucopyranose residues.

As reported previously, the neutral monosaccharides of the tuberous roots of *Biebersteinia multifida* DC consist of three glucans, A, B, and C, which have been isolated in the individual form [1]. In the present paper we give information on the investigation of their structures.

Glucans A, B, and C were subjected to oxidation with $NaIO_4$ and CrO_3, and the results of the analysis are given in Table 1. Glucans A, B, and C are low-molecular-mass polymers and therefore, as can be seen from Table 1, they were oxidized in 4-9 days. For each anhydro unit in glucans A, B, and C 1.7-1.9 mole of oxidant was consumed and about 0.9 mole of formic acid was produced. Glycerol and glucose were detected in the corresponding ratios in the products of Smith degradation [2] (Table 1).

The consumption of a large amount of oxidant and the formation of a considerable amount of glycerol showed the presence of 1 → 3 bonds between the monosaccharides and also the pyranose form of the glucose residues in the chains of the glycans. The detection of glucose in the oxidation products presupposes the presence of branching at the C-3 atoms of glucopyranose residues in the chains of the glucans.

More detailed information on the structures of the glucans was given by the exhaustive Hakomori methylation of A, B, and C [3]. Glucans A, B, and C were methylated twice, each separately. The IR spectra of the completely methylated A, B, and C contained no absorption bands of hydroxy groups. The permethylates of A, B, and C were subjected to formolysis and hydrolysis. The products given in Table 2 were identified in the hydrolysis products by TLC and GLC with authentic samples.

The detection of 2,4-di-O-Me-glucopyranose confirmed the results of periodate oxidate oxidation concerning C-3 atoms in the chains of glucans A, B, and C. The presence of 2,3,4-tri-O-Me-glucopyranosein the hydrolysates of the permethylates of A, B, and C showed that glucans A, B, and C contained mainly 1 → 6-bound glucopyranose residues.

The results of a mass-spectrometric study of the permethylates isolated individually to determine the distribution of the methyl groups (Table 2) completely confirmed the results of periodate oxidation and methylation.

The high positive specific rotations of compounds A, B, and C and absorption bands at 860 cm^{-1} in the IR spectra showed the presence of α-glycosidic bonds between the monosaccharide residues, which was likewise confirmed by the results of the oxidation of the completely acetylated A, B, and C with chromic anhydride (Table 1). It is known that under these conditions only monosaccharide residues linked by β-glycosidic bonds undergo oxidation [4]. Free glucose residues were detected in the products of the chromic acid oxidation of A, B, and C, i.e., the glucans did not undergo oxidation.

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TABLE 1. Results of the Analysis of the Periodate and Chromic Acid Oxidation of Glucane A, B, and C of B. multifida

Glucan	Time of oxidation, days	Consumption of $NaIO_4$, mole	HCOOH produced, mole	Products of hydrolysis after periodate oxidation, mole		Products of hydrolysis after chromic acid oxidation
				glycerol	glucose	
A	9	1,9	0,9	11	1	Glucose
B	7	1,8	0,9	12	1	Glucose
C	4	1,7	0,8	5	1	Glucose

TABLE 2. Results of the Analysis of Partially Methylated Polyol Acetates from Glucans A, B, and C of B. multifida

Sugar component	Time, min	Molar ratio			Main mass-spectral fragments, m/z	Type of bond
		A	B	C		
2,3,4,6-Tetra-O-Me-Glcp	1.0	1,6	2,0	2,1	43,45,71,87,101,117, 129,145,161,205	Glcp-(1→
2,3,4-Tri-O-Me-Glcp	2,05	9,6	10,3	3,2	43,87,99,101,117,129, 161,189	→6)-Glcp-(1→
2,4-Di-O-Me-Glcp	3,60	1,0	1,0	1,0	43,87,117,129,189	→3,6)-Glcp-(1→

The neutral polysaccharides of the tuberous roots of B. multifida consist of three weakly bound glucans, A, B, and C. In the chains of glucans A, B, and C, the monosaccharides were linked with one another by α-1 → 6 bonds and there is branching at C-3 atoms of glucopyranose residues.

EXPERIMENTAL

TLC was conducted on Silufol UV-254 plates in the following solvent systems: 1) benzene–acetone (2:1); 2) chloroform–methanol (9:1); 3) methyl ethyl ketone–1% NH_4OH (30:1).

For PC we used Filtrak FN-3, 11, and 12 papers in the following solvent systems: 4) butan-1-ol–pyridine–water (6:4:3); and 5) propanol–ethyl acetate–water (7:2:1).

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

GLC was conducted on a Chrom-1 chromatograph with a flame-ionization detector under the following conditions: stainless column (0.3 × 200 cm), 5% of silicone XE-60 on Chromaton NAW-0.200-0.255 mm, 210°C, carrier gas helium 60 ml/min for the polyol acetates.

The mass spectra of the substances were taken on an MKh-1310 instrument with direct introduction of the sample into the source at a temperature of the ionization chamber of 200°C and of the evaporator bulb of 180-250°C. The collector current was 60 μA.

Periodate Oxidation and Smith Degradation. Glucans A, B, and C (0.05 g each) were each dissolved in 25 ml of water, and each solution was treated with 5 ml of 0.25 M sodium periodate solution and was left at +5°C. Aliquots of 1 ml were taken every day and were treated with a 0.01 N solution of sodium thiosulfate. After 9 days, the consumption of sodium periodate amounted to 1.9 mole for glucan A, after 7 days to 1.8 mole for B and after 4 days to 1.7 mole for C, and these amounts did not change further. The formic acid liberated in the course of the reaction was titrated with 0.01 N NaOH and it was calculated that its amount was 0.9 mole for A and B and 0.8 mole for C. After the decomposition of the periodate with ethylene glycol, the products were diluted with water and to each was added 0.1 g of $NaBH_4$ and the mixtures were left overnight. The solutions were treated with KU-2 cation-exchange resin (H^+), and the filtrates were evaporated to dryness, and then the boric acid residues were eliminated by distillation with methanol. Each of the dry residues was hydrolyzed in 3 ml of 1 N H_2SO_4 at 100°C for 8 h. Mainly glycerol, and also glucose, were also detected in the products of the hydrolysis of glucans A, B, and C by PC (system 4, revealing agents 1 and 2) and GLC.

Methylation of Glucans A, B, and C. Compounds A, B, and C (0.1 g each) were methylated twice by the Hakomori method [3]. The methylated products were extracted with chloroform, and the chloroform extracts were evaporated and precipitated with petroleum ether. The completeness of methylation was checked by TLC (systems 2 and 3; spray reagent 1) and by IR

spectroscopy (absence of absorption bands of hydroxy groups at 3200-3600 cm^{-1}). The yields of the permethylates of the glucans were as follows: A – 0.08 g, –O–CH_3 – 42.5%, $[\alpha]_D^{23}$ + 86° (c 0.8; acetone); B – 0.082 g, –O–CH_3 – 41.7%, $[\alpha]_D^{23}$ + 118° (c 0.82; acetone); C – 0.074 g, –O–CH_3 – 42.0%, $[\alpha]_D^{23}$ + 103° (c 0.74; acetone).

Formolysis and Hydrolysis of the Permethylates of A, B, and C. Each glucan permethylate (0.05 g) was boiled in the water bath with 5 ml of 85% formic acid for 1 h, and the mixtures were cooled and evaporated. Then 5 ml of 0.5 N H_2SO_4 was added to each residue and hydrolysis was carried out at 100°C for 12 h. The hydrolysates were worked up in the usual way. The products were studied by TLC (system 1, revealing agent 1), and 2,3,4,6-tetra-, 2,3,4-tri-, and 2,4-di-O-Me-glucoses were detected in all the samples. The partially methylated polyol acetates were obtained and were studied by the GLC method.

Isolation of the Methylated Sugars. The hydrolysates of the permethylates were separated on Silufol UV-254 plates in system 1, the zones corresponding to the methylated sugars were cut out and extracted with chloroform, and the extracts were evaporated. This gave chromatographically individual products: 2,3,4,6-tetra-, 2,3,4,tri-, and 2,4-di-O-Me-D-glucopyranoses (12, 16, and 8 mg, respectively). Each product was studied by mass spectrometry in the form of acetates of the partially methylated polyols.

Acetylation of Glucans A, B, and C. Separately, 0.1-g samples of glucans A, B, and C, were dissolved in 5-ml portions of formamide, and to each solution was added 7 ml of anhydrous pyridine and, dropwise, 7 ml of acetic anhydride, and the reaction mixtures were stirred for 5 days. Then each was precipitated with 0.5 liter of ice water, and the precipitates were separated by centrifugation, washed with ice water and dried in vacuum over P_2O_5. The yield of the peracetate of A was 0.146 g, B 0.142 g, and C 0.141 g. The IR spectra lacked absorption bands of hydroxy groups, while bands at 1750 and 1240 cm^{-1} were well-defined.

Oxidation of the Peracetates A, B, and C with Chromium Trioxide. Three flasks were each charged with 0.2 g of chromium trioxide, which was dissolved in 5 ml of glacial acetic acid, and to these solutions were added 0.1 g of peracetates A, B, and C, respectively, and the reaction mixtures were heated at 50°C for 4 h. They were then diluted with water and extracted with chloroform, and the extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residues were each dissolved in 1 ml of 1 N H_2SO_4 and were hydrolysed on the boiling water bath for 12 h. The hydrolysates were worked up in the usual way, and PC (systems 4 and 5; revealing agent 1) and GLC revealed free glucose residues.

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