POLYSACCHARIDES OF *Polygonatum*.

IV. A STUDY OF THE STRUCTURE OF THE GLUCOMANNAN OF *Polygonatum severzovii*

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The structure of the glucomannan established on the basis of the results of methylation, chromium trioxide oxidation of the acetylated glucomannan, and partial hydrolysis, is given. The partial hydrolysis of the glucomannan gave a series of oligosaccharides — 4-O-β-D-glucopyranosyl-D-mannopyranose, di-, tri-, and tetrasaccharides, and β-1→4-bound D-mannooligosaccharides — the structures of which follow from the results of hydrolysis, periodate oxidation, methylation, and mass spectrometry. A structure is proposed for the repeating unit.

We have previously [1] described the isolation and fractionation of the acetylated glucomannan severan from the rhizomes of *P. severzovii* Regel.

To determine the nature of the substitution of the monosaccharide residues in the glucomannan chain, we methylated it by Hakomori's method [2]. The permethylate of the glucomannan was subjected to formolysis and to hydrolysis. The hydrolysis products were studied by TLC and GLC with markers. 2,3,4,6-Tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-mannose, and trace amounts of di-O-methylhexose were detected. The demethylation of the last-mentioned compound [3] gave mannose.

The ratio of 2,3,6-tri-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-mannose (1:15.4) coincides with the ratio of the free sugars in a hydrolysate of the initial glucomannan. The presence of 2,3,4,6-tetra-O-methyl-D-mannose shows that the glucomannan chain has a mannopyranose residue at the nonreducing end. The negative specific rotation of severan permethylate and absorption bands at 890 cm^{-1} in the IR spectrum show the presence of a β-glycosidic bond between the monosaccharide residues. This hypothesis is also confirmed by the oxidation of glucomannan peracetate with chromium trioxide. It has been shown previously [4] that in such a case only the monosaccharide residues bound by a β-glycosidic bond undergo oxidation. The product of the oxidation of acetylated severan contained no monosaccharides, which shows a β bond between the monosaccharide residues.

Thus, it follows from the facts given that the polysaccharide has basically a linear structure with β-(1→4) bonds between D-mannopyranose and D-glucopyranose residues.

To determine the sequence of monosaccharide residues in the polysaccharide chain and the structure of the main unit we used partial hydrolysis. From a hydrolysate, with the aid of preparative paper chromatography, we isolated four oligosaccharides (A-D). The structures of the oligosaccharides were established by the methods of complete and partial hydrolysis before and after reduction with sodium tetrahydroborate, methylation, periodate oxidation, and mass spectrometry. The following oligosaccharides were identified: 4-O-β-D-glucopyranosyl-D-mannopyranose (A), 4-O-β-D-mannopyranosyl-D-mannopyranose (B), O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose (C), and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose (D).

The results obtained permit the conclusion that the glucomannan from *P. severzovii* has a linear structure with the repeating unit:

$$[\text{Manp}(1\rightarrow4)\text{-O-}\beta\text{-}D\text{-Manp}(1\rightarrow4)\text{-O-}\beta\text{-}D\text{-Manp}(1\rightarrow4)\text{-O-}\beta\text{-}D\text{-Manp-}(1\rightarrow4)\text{-O-}\beta\text{-}D\text{-Glcp}(1\rightarrow4)\text{-O-}\beta\text{-}D\text{-Manp....}]$$

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EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. Paper chromatography was performed on FN 3,11 paper by the descending method using the following system (by volume): 1) butan-1-ol—pyridine—water (6:4:3). Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates and on plates with KSK silica gel in the following systems: 2) methyl ethyl ketone—1% NH_3 (30:1); 3) chloroform—methanol (9:1). To identify the spots we used the following reagents: 1) aniline hydrogen phthalate (at 105-110°C for 10-15 min); 2) periodate—$KMnO_4$—benzidine; and 3) concentrated H_2SO_4. The GLC of the samples was carried out on Tsvet-101 instrument with a flame-ionization detector under the following conditions: steel column (0.3 × 200 cm), Chromaton N-AW, 0.200-0.250 mm, impregnated with 5% of Silicone XE-60; carrier gas helium, 60 ml/min; temperature 210-270°C. The acetate of the aldononitriles and the acetates of the polyols corresponding to the methylated sugars were obtained as described by Ovodov [7]. The oligosaccharides were hydrolyzed with 1 N H_2SO_4 at 100°C for 4 h. They were reduced with $NaBH_4$. The degree of polymerization of the oligosaccharides was determined by gas—liquid chromatography from the ratio of the reducing sugars after the reduction of the oligosaccharides. The oligosaccharides were methylated by Hakomori's method [2] and were subjected to periodate oxidation with 0.05 M $NaIO_4$ at 4°C for 60 h.

Methylation of Severan. The Hakomori methylation of 0.2 g of the glucomannan gave 0.11 g of glucomannan permethylate having no absorption in the OH region (3200-3600 cm^{-1}). $[\alpha]_D^{22}$ −24.4° (c 0.9; acetone). The glucomannan permethylate (0.01 g) was subjected to formolysis and hydrolysis, and the products were studied by TLC (system 2, revealing agent 1) and GLC. The following set of methylated sugars was detected: 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-mannose, and a di-O-methylhexose, with R_f 1.01,* 0.76, 0.58, and 0.28, respectively.

Demethylation of the Di-O-methylhexose. The di-O-methylhexose (0.005 g) was demethylated by a handbook method [3]. The demethylation products were identified by PC (system 1, revealing agent 1), mannose being detected.

Oxidation of Severan with Chromium Trioxide. A solution of 0.044 g of the substance in 15 ml of formamide was treated with 7 ml of anhydrous pyridine and then 7 ml of acetic anhydride was added dropwise and the mixture was stirred for 5 days. The acetate was precipitated in ice-containing distilled water. The precipitate was separated off, washed with methanol, and dewatered with acetone. The yield was 0.08 g. The severan acetate (0.08 g) was added to a solution of 0.3 g of CrO_3 in 7 ml of glacial acetic acid and the mixture was heated at 50°C for 4 h. Then it was diluted with water and extracted with chloroform, and the chloroform extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in 1 ml of 1 N H_2SO_4 and was hydrolyzed in the boiling water bath for 16 h, the hydrolysate being worked up by the usual method. No hexoses were detected by PC (system 1, revealing agent 1).

Partial Hydrolysis of Severan. A solution of 1.0 g of the substance in 125 ml of water was treated with 41.6 ml of 2 N H_2SO_4 and the mixture was kept at 90°C for 2 h. Then it was neutralized with $BaCO_3$, treated with KU-2 cation-exchange resin (H^+), evaporated to a syrup, and separated preparatively by PC (system 1, revealing agent 1). Free mannose and glucose were isolated, together with four oligosaccharides (A-D) with R_m 0.82 (A), 0.61 (B), 0.22 (C), and 0.07 (D).

4-O-β-D-Glucopyranosyl-D-mannopyranose. The oligosaccharide (0.01 g) was subjected to complete acid hydrolysis, and then PC (system 1, revealing agent 1) showed the presence of D-glucose and D-mannose in a ratio of 1:1. The oligosaccharide (0.01 g) was methylated by Hakomori's method [2] and in a hydrolysate of the permethylate 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,6-tri-O-methyl-D-mannose were identified in a ratio of 1:1.

4-O-β-D-Mannopyranosyl-D-mannopyranose. 0.01 g of the substance was subjected to complete acid hydrolysis and to PC (system 1, revealing agent 1); mannose was detected. The oligosaccharide (0.01 g) was reduced with $NaBH_4$, and mannitol and mannose were detected in the hydrolysate (1:1); 0.053 g of the substance was subjected to Hakomori methylation, and in a hydrolysate of the permethylate 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-mannose were detected in a ratio of 1:1.

*As in Russian original — Publisher.

O-β-D-Mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose. When 0.01 g of the substance was subjected to complete acid hydrolysis and the products were investigated by PC (system 1, revealing agent 1), mannose was found. When 0.01 g of the substance was reduced with $NaBH_4$, mannitol and mannose were identified in the hydrolysate in a ratio of 1:2. The oligosaccharide (0.01 g) was methylated by Hakomori's method [2], and in a hydrolysate of the permethylate 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-mannose were detected in a ratio of 1:2. The oligosaccharide (0.005 g) was oxidized with 0.05 M $NaIO_4$ at 4°C for 60 h. The periodate was decomposed with ethylene glycol, and then 0.01 g of $NaBH_4$ was added to the mixture and reduction was carried out for 3 h. The resulting solution was treated with KU-2 cation-exchange resin (H^+), the filtrate was evaporated to dryness, and the residue was evaporated several times with methanol. In a hydrolysate PC (system 1, revealing agent 2) and GLC showed the presence of glycerol and of erythritol in a ratio of 1:2.

O-β-D-Mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose. When 0.01 g of the oligosaccharide was subjected to complete acid hydrolysis and the products were investigated by PC (system 1, revealing agent 1), mannose was detected. When 0.01 g of the substance was reduced with $NaBH_4$ and the product was hydrolyzed, mannitol and mannose were found in a ratio of 1:3. When 0.055 g of the oligosaccharide was subjected to Hakomori methylation [2] and the permethylate was hydrolyzed, 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-mannose were found in the hydrolysate in a ratio of 1:3. The substance (0.0087 g) was oxidized with 0.05 M $NaIO_4$ at +4°C for 60 h and the products were worked up by the method described above. Paper chromatography of the hydrolysate (system 1, revealing agent 2) and GLC showed the presence of glycerol and erythritol in a ratio of 1:3.

An N-p-Tolylglycosamine from the Oligosaccharide. An acetylated N-p-tolylglycosamine was obtained from 0.0035 g of the substance by the method of Usov and Barbakadze [5]. The mass spectrum of the product showed the peak of the molecular ion with m/z 1301 and also the peaks of ions with m/z 378, 666, and 969 arising as the result of the cleavage of the bonds between the monosaccharide residues and relating to the reducing end, and the peaks of ions with m/z, 331, 619, and 905, corresponding to the nonreducing end.

The results obtained correspond to those given in the literature [6].

SUMMARY

The structure of a glucomannan from *Polygonatum severzovii* Regel. has been studied with the aid of methylation, chromium trioxide oxidation, and partial hydrolysis. The results obtained show that severan has a linear chain of β-(1→4)-linked D-glucopyranose and D-mannopyranose residues. A structure is put forward for the repeating unit.

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