POLYSACCHARIDES OF *Polygonatum*

I. ISOLATION AND CHARACTERIZATION OF POLYSACCHARIDES FROM *Polygonatum sewerzowii*

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Continuing an investigation of polysaccharides of plants of the family Liliaceae [1, 2], we have begun the study of plants of the genus *Polygonatum* Adans. There are reports in the literature on the isolation of the polysaccharides odoratan and fructan from the rhizomes of *P. odoratum* Druce var. *japonicum* Hara [3, 4] and of falcatan from *P. falcatum* A. Grav. [5, 6] cultivated in Japan.

The genus *Polygonatum* is represented in the USSR by 18 species [7], of which two — *P. sewerzowii* Regel. and *P. roseum* (Lab.) Kunth — grow in the territory of Central Asia [8].

We have studied the polysaccharide of the rhizomes of *P. sewerzowii* collected in the vegetation phase on April 5, 1977, at Galvasae, Bostanlyk region, Tashkent oblast. The isolation and fractionation of the water-soluble polysaccharides was carried out in accordance with Scheme 1.

The air-dry raw material was boiled with ethanol to eliminate colored substances and low-molecular-weight compounds. The ethanolic extract contained mannose, glucose, fructose, and sucrose. Exhaustive extraction of the residue of the raw material with water at room temperature gave an aqueous extract which, on the addition of ethanol, formed a precipitate (fraction A). The supernatant liquid, on evaporation and reprecipitation with ethanol, gave a precipitate (fraction B) and an ethanolic-aqueous solution (fraction C). The latter contained galactose, mannose, glucose, fructose, sucrose, raffinose, and oligo- and polysaccharides consisting of fructose and glucose. The plant residue after the aqueous extraction was heated with water. As a result of the usual working up of the extract, a fraction of polysaccharide D was obtained which, on hydrolysis gave rhamnose, arabinose, mannose, and galactose in a ratio of 1:5.7:21.4:11.2, and traces of fructose and glucose.

On complete acid hydrolysis of polysaccharides fraction A, arabinose, mannose, glucose, and galactose were identified in a ratio of 1.5:13.9:1.6:1, together with traces of uronic acids. A solution of fraction A was separated by passage through a column of DEAE-cellulose. The elution curve had two peaks (Fig. 1). The polysaccharide corresponding to peak I, eluted with water, formed the bulk (62.6% of fraction A). On its acid hydrolysis, mannose, glucose, and a small amount of galactose were detected.

The polysaccharides eluted by 1 M sodium acetate (9%) consisted of arabinose, mannose, glucose, and galactose in a ratio of 1:29.1:4.6:11, with traces of uronic acid. The further separation of the polysaccharides obtained from the aqueous eluate was performed by fractional precipitation with ethanol. Three fractions were obtained (yields 0.46, 0.15, and 0.08 g, respectively), of which fraction 1 formed the main part (58%). According to the results of sedimentation analysis, this water-stable polysaccharide was homogeneous and had a molecular weight of 59,000. Its acid hydrolysis formed D-glucose and D-mannose. The latter was isolated and identified in the form of crystalline derivatives. The ratio of the monosaccharides was 1:15.4 and did not change when the polysaccharide was purified by various methods. Consequently, it was a glucomannan. It differed from odoratan and falcatan [2, 4] in its composition and properties, and we have called it severan.

Thus, the scheme of isolating the polysaccharides that was used enabled us to obtain a glucomannan, and this was studied further with the aid of IR spectroscopy and Smith degradation.

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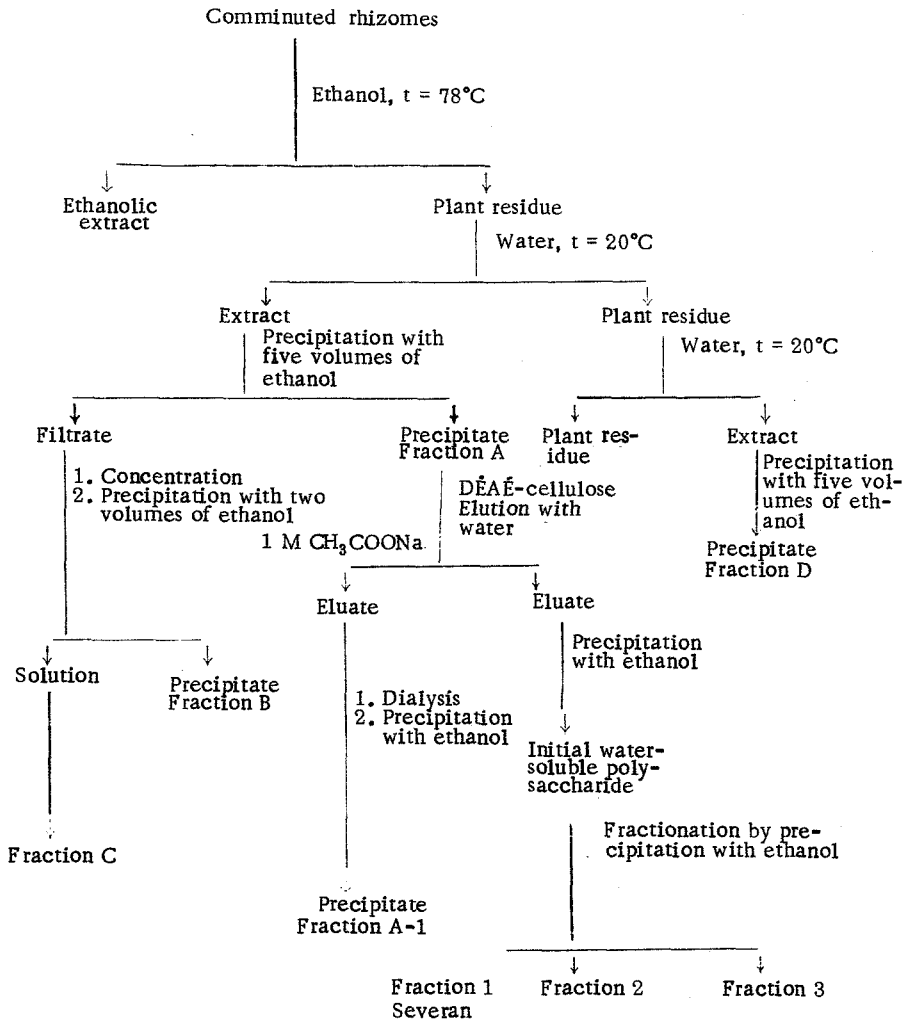

Scheme 1. The isolation and fractionation of the polysaccharides of *Polygonatum sewerzowii*.

The IR spectra of severan and its deacetylated derivative obtained via the copper complex (DAS) contained absorption bands at 890 cm^{-1} (β-glycosidic bond) and 815 cm^{-1} (pyranose ring). In addition, the spectrum of severan had absorption bands at 1740 and 1250 cm^{-1} (ester group), which were absent from DAS. Assuming that these bands are due to the presence of O-acyl groups, we subjected severan to hydrolysis and methanolysis. In the hydrolyzate acetic acid was detected by paper chromatography (PC) and in the methanolyzate a single peak corresponding to methyl acetate was found by GLC (conditions C).

Severan was oxidized with periodic acid, and the resulting product was reduced with sodium tetrahydroborate and was hydrolyzed. The presence in the hydrolyzate of erythritol and mannose in a ratio of 14.5:1 showed a 1 → 4 bond between the hexose residues and the presence of branching through a 1 → 3 or 1 → 2 bond in some of the mannose residues.

Thus, it may be concluded that severan belongs to the β-1 → 4-bound glucomannans and differs from the known ones by the ratio of the components and the structure of the molecule.

EXPERIMENTAL

The solutions were separated in vacuum at 40 ± 5°C. For descending chromatography we used Filtrak FN 7 and 11 papers in the following solvent systems (by volume): 1) butan-1-ol—pyridine—water (6:4:3); 2) phenol—butan-1-ol—acetic acid—water (20:20:8:40). The zones of the reducing sugars were detected with aniline hydrogen phthalate (10 min at 105-110°C), and those of the polyols with a 1% solution of permanganate-benzidine after periodate oxidation.

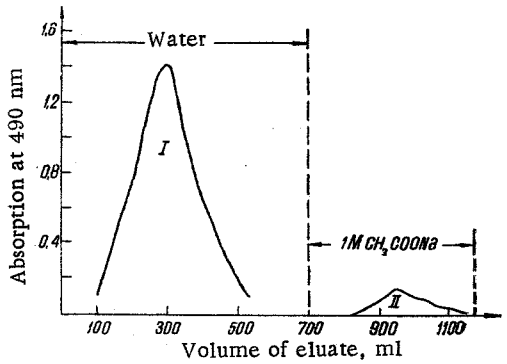

Fig. 1

The GLC of the samples was performed on a Tsvet-101 instrument with a flame-ionization detector and a stainless-steel column (200 × 0.3 cm). Conditions of chromatography: A) 5% of silicone XE-60 on Chromaton NAW, 0.200-0.250 mm, 210°C, air, 300 ml/min, H_2 and helium 60 ml/min each, using the aldononitrile acetates; B) 3% of poly(neopentyl glycol adipate) on Chromaton NAW-DMA (0.125 × 0.160 mm), 210°C, helium, 60 ml/min, for the polyol acetates; and C) column 100 × 0.3 cm, 5% of neopentyl glycol succinate + 1% of poly(ethylene glycol adipate) on Chromaton (50°C, helium, 30 ml/min). The IR spectra of the samples were recorded on a UR-20 instrument in tablets with KBr.

The complete hydrolysis of polysaccharide fractions A-D (see Scheme 1) was carried out with 2 N H_2SO_4 at 100°C for 8 h, followed by neutralization with $BaCO_3$ and evaporation to 1 ml. Part of the hydrolyzate was analyzed by PC and another part was used for obtaining the aldononitrile acetates [9] for GLC analysis.

Isolation of the Polysaccharides. Air-dry, comminuted, and sieved (1-mm sieve) raw material (340 g) was boiled with 3 liters of ethanol for 1 h, and the extract was filtered and concentrated to a syrup, in which mannose, glucose, fructose, and sucrose were found by PC in systems 1 and 2. The plant residue was extracted with 12 liters of water for 3 h and the extract was filtered, and the treatment was repeated twice more (3.5 liters of water each time). The extracts were combined, evaporated to 1/2 volume, and poured into five volumes of ethanol. The slimy fibrous precipitate that deposited was separated off, washed with ethanol and with acetone, and dried in vacuum over P_2O_5. Yield 13.56 g (fraction A). The mother solution was evaporated to a syrup and was poured into two volumes of ethanol and the precipitate was separated off, washed, and dried. Yield 2.69 g (fraction B). The ethanolic mother liquor was concentrated to a syrup, which was dissolved in water, and the solution was treated with activated carbon, reconcentrated to the state of a viscous syrup, and triturated with acetone to give a powder. Yield 60.11 g (fraction C).

The plant residue was treated with water twice (1 liter at 100°C for 6 h each time). The supernatant was concentrated and was poured into five volumes of ethanol. The precipitate that deposited was separated off and dried. Yield 1.2 g (fraction D).

Chromatography on DEAE-Cellulose. DE-32 DEAE-cellulose (60 g) was washed successively with 1 liter of 0.5 N HCl, water to neutrality, 1 liter of 0.5 N NaOH, water, 1 liter of 1 M $(NH_4)_2CO_3$, and again with water. Then it was placed in a column (35 × 4 cm), an aqueous solution of 0.2 g of the polysaccharide (fraction A) was deposited on it, and elution was carried out with water and then with 1 M CH_3COONa. Fractions with a volume of 50 ml were collected, and the polysaccharides in 1-ml samples of them were detected by the phenol/sulfuric acid method (see Fig. 1). The fractions corresponding to peak I (100-600 ml) were combined, evaporated, and poured into four volumes of ethanol. The snow-white precipitate was washed with ethanol and acetone and was dried. The fractions corresponding to peak II (800-1050 ml) were dialyzed against water, concentrated, and poured into three volumes of ethanol. The precipitate that deposited was separated off, washed with ethanol and acetone, and dried in vacuum. From 2 g of fraction A, 1.15 g of (I) and 0.18 g of (II) were obrained.

Fractional Precipitation with Ethanol. With vigorous stirring, 400 ml of ethanol was added dropwise to a solution of 0.8 g of the polysaccharide (fraction 1) in 200 ml of water. The precipitate that deposited was separated off by centrifuging and was washed with ethanol, acetone, and ether and dried to constant weight. Yield 0.46 g (fraction 1, severan). The

supernatant liquid was treated with another 400 ml of ethanol and the resulting precipitate was treated similarly, with a yield of 0.15 g (fraction 2). The ethanolic mother solution was evaporated to a syrup and precipitated with four volumes of ethanol, and the precipitate was dried, yield 0.08 g (fraction 3).

In a hydrolyzate of severan D-mannose and D-glucose were detected (PC in system 1). Their ratio was 15.4:1 (according to GLC, in the form of the polyol acetates, conditions B, and in the form of the aldononitrile acetates, conditions A).

The severan hydrolyzate was treated with phenylhydrazine [10], and D-mannose phenylhydrazone was obtained. mp 189-190°C.

The O—Ac groups in severan were identified by a method described previously [11].

Precipitation with Fehling's Solution. With stirring, 2.5 ml of Fehling's solution was added to a solution of 0.21 g of severan in 80 ml of water. The precipitate was separated off by centrifuging and was washed successively with 80% and 5% CH_3COOH and with water, after which it was dehydrated with ethanol and was dried. The yield of DAS was 0.17 g. It formed a white fibrous powder insoluble in water and soluble in NaOH and HCOOH.

In the cold, 20 mg of DAS was dissolved in 0.5 ml of 72% sulfuric acid and the solution was then diluted with water to a final concentration of the acid of 2 N and hydrolysis was carried out at 95-100°C for 10 h. The hydrolyzate was neutralized with $BaCO_3$ and was treated with KU-2 cation-exchange resin (H^+). Only mannose and glucose were detected in the syrup by PC and GLC, in a ratio of 15.4:1.

Periodate Oxidation of Severan. A mixture of 50 mg of severan and 16 ml of 0.05 M $NaIO_4$ was kept at +5°C for 15 days. The total consumption of periodate was 1 ml per monosaccharide residue. The product was dialyzed (after the destruction of the excess of periodate with ethylene glycol) and then 100 mg of $NaBH_4$ was added, the mixture was left overnight, and the resulting solution was treated with KU-2 anion-exchange resin (H^+), after which the filtrate was evaporated to dryness and was then evaporated with ethanol several times. In the hydrolysis product (2 N H_2SO_4 at 100°C, 6 h) erythritol (full acetate, column B) and mannose in the form of the aldononitrile acetates (column A) were detected by PC and GLC.

SUMMARY

The water-soluble polysaccharides of the rhizomes of the *Polygonatum sewerzowii* Regl. have been studied. A method has been developed for separating the polysaccharide fraction. A new native acetylated glucomannan has been isolated which has been called severan and consists of manno- and glucopyranose residues linked with one another by β-1 → 4 bonds in a ratio of 15.4:1.

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