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POLYSACCHARIDES OF Polygonatum.

VII. A GLUCOFRUCTAN FROM P. sewerzowii

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A glucofructan has been isolated from the rhizomes of Polygonatum sewerzowii Bunge and has been called pseverin. According to its chemical and spectral characteristics, pseverin is a polysaccharide containing both inulin, 2 → 1, and leven, 2 → 6, glycosidic bonds. The problem of the correlation between the molecular structure of a glucofructan and its supermolecular organization has been considered. X-radiographic and electron-microscope studies have been made of pseverin and its acetate. It has been established that on precipitating from solution pseverin forms globular particles which aggregate to give elongated necklace-like formations. Fine lamellar structures are characteristic for the acetate.

We have previously reported the isolation of water-soluble polysaccharides from the rhizomes of Polygonatum sewerzowii Ragel [1]. After the water-soluble polysaccharide (WSPS) had been precipitated with ethanol from the aqueous ethanolic filtrate, we obtained the combine polysaccharides with a yield of 17.6% on the air-dry raw material. The polysaccharides were dialyzed against distilled water. The dialysate was found to contain glucose, fructose, sucrose, and fructooligosaccharides. The undialyzed part remaining within the membrane was evaporated to a syrup and, by trituration with acetone, was converted into a powder. A hydrolysate of the latter was found by PC (system 1, revealing agents 1 and 2) to contain glucose and fructose. Consequently, this polysaccharide was a glucofructan; it has been called pseverin. A determination of the amounts of monosaccharides in the polysaccharide by the Bertrand–Kolthoff method [2] showed that it contained 96% of fructose and 4% of glucose.

Pseverin is a white amorphous powder readily soluble in water, $[\alpha]_D^{22}$ 30° (c 0.4; water). On gel filtration through Sephadex G-50, pseverin proved to be homogeneous. Its IR spectrum contained absorption bands at 820 and 940 cm^{-1}, which are characteristic for glucofructans of the inulin type and at 860 cm^{-1}, which are characteristic for levan. Consequently, pseverin contains 2 → 1 and 2 → 6 bonds.

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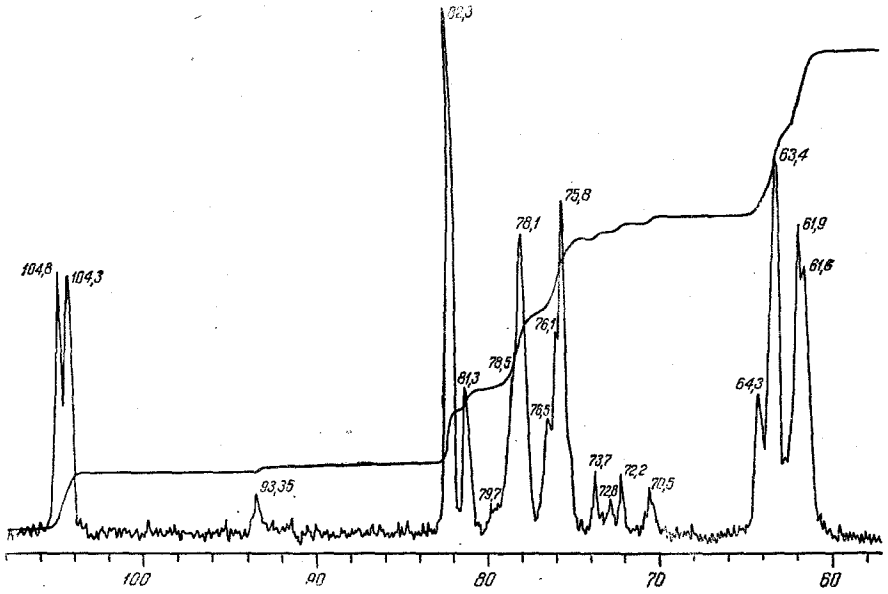

Fig. 1. ^{13}C NMR spectrum of pseverin.

The results of IR spectroscopy and the negative specific rotation showed the predominance of β-glycosidic bonds and the ease of acid hydrolysis indicated the furanose form of the D-fructose. When pseverin was subjected to periodate oxidation, for each mole of anhydro unit 1.06 moles of $NaIO_4$ was consumed, with the formation of 0.0389 moles of HCOOH. In the products of Smith degradation [3], glycerol was found by PC (system 1, revealing agent 4) and GLC, which indicated the presence of 2 → 1 or 2 → 6 bonds between the hexose residues.

The methylation of pseverin [4] gave a permethylate, which was subjected to methanolysis. In the resulting methanolysate 2,3,4,6-tetra-O-methyl-D-glucose, 1,3,4,6-tetra-O-methyl-D-fructose, 3,4,6-tri-O-methyl-D-fructose, and 1,3,4-trimethyl-D-fructose were detected as the main components by TLC (systems 3 and 4; revealing agents 1, 2, and 3) and GLC (conditions B, in the form of methyl glycosides). The identification of 3,4,6-tri-O-methyl-D-fructose indicated that 2 → 1 bonds were present in the glucofructan, and the predominance of 1,3,4-tri-O-methyl-D-fructose showed the presence of 2 → 6 bonds between the fructofuranose residues [5].

Thus, in pseverin the monosaccharide residues are bound by 2 → 1 and 2 → 6 bonds and the glucose is present at the end of the polymer chain.

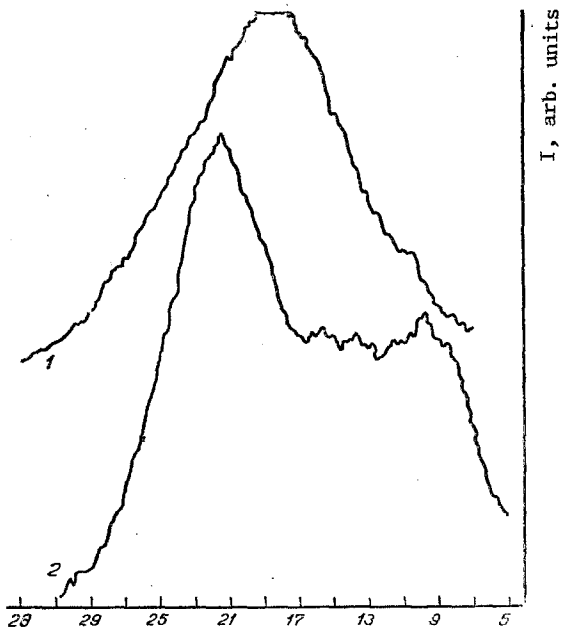

Fig. 2. Diffractograms: 1) the glucofructan (pseverin); 2) pseverin acetate.

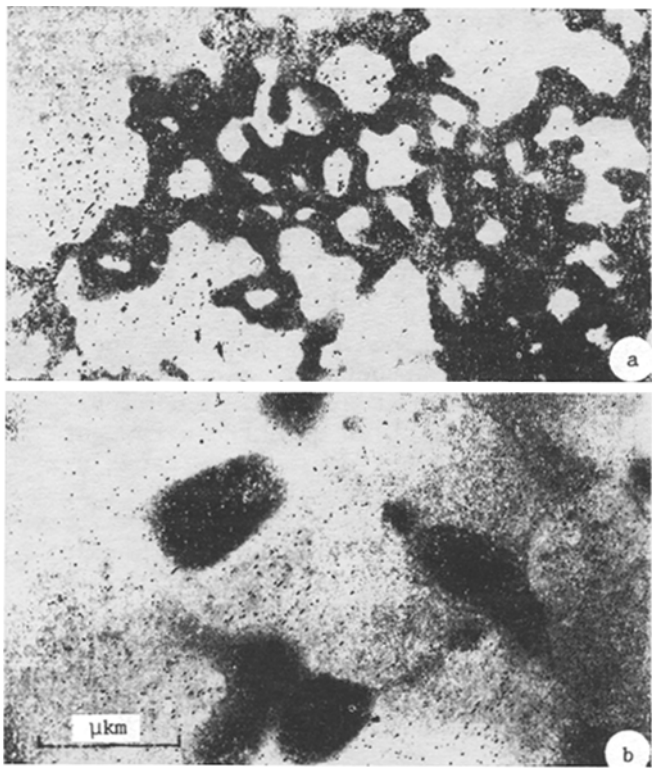

Fig. 3. Electron micrographs of preparations of: a) pseverin; b) pseverin acetate.

The results obtained confirmed those obtained by a study of pseverin by ^{13}C NMR spectroscopy. The spectrum (Fig. 1) showed the chemical shifts characteristic for fructofuranose units linked by (2 → 1)- and (2 → 6)-glycosidic bonds. The quantitative ratio of (2 → 1) and (2 → 6) bonds calculated from the integral intensities of the ^{13}C NMR signals was 2.7:1. The heterogeneity of pseverin in relation to the types of bonds followed from the presence in the spectrum of specific signals from residues present at the junctions of units with different types of bonds.

Below we give the chemical shifts of the carbon atoms in the ^{13}C NMR spectrum of pseverin:

Residues	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
(2 → 1)-bound fructofuranose units	61,9	104,3	78,1	75,8	82,3	63,4
(2 → 6)-bound fructofuranose units	61,6	104,8	78,5	76,1	81,3	64,3
α-D-glucopyranose units	93,35	72,8	73,7	70,5	72,2	61,6

The glucose was present at the reducing end of the polymer chain and was attached to C-2 of a fructofuranose unit, as was also shown by the chemical shift of the C-1 atom of the α-glucopyranose unit (93.35 ppm) which is characteristic for this type of linkage.

Thus, the pseverin isolated from the rhizomes of P. sewerzowii has in its chain a combination of two types of bonds (the inulin and the levan types) and differs from the glucofuran of Allium [6] by the ratio of units with different types of bonds.

X-radiographic and electron-microscope investigations have been performed of pseverin and its acetate (Fig. 2). The x-ray diffractogram of pseverin consists of a curve with a single broad maximum at 2θ = 18°, which shows the amorphous nature of this polysaccharide. The diffractogram of the acetate characterizes a more ordered structure, having two maxima at 2θ = 9.5° and 21.5°, which correspond to predominant distances of 9.2 and 4.08 Å, or 0.92 and 0.41 nm. Thus, in at least two directions the chains in the glucofructan acetate are arranged in a fairly ordered manner.

The electron-microscope investigations also indicated appreciable differences in the supermolecular structures of these substances. On precipitation from solution, pseverin forms globular particles which aggregate to form long necklace-like formations (Fig. 3, a).

Characteristic for the acetate are thin lamellar structures in which the chains are probably located in ordered fashion in two main directions and are disordered in the direction perpendicular to the plane of the lamella (Fig. 3b).

On the basis of this glucofructan and the glucomannan that we have studied previously [7], and also in light of the literature information on the structure of inulin [8], levan, and other polysaccharides [9], it is possible to draw a number of conclusions concerning the interrelationship between the molecular structure of a polysaccharide and its supermolecular organization. It is known that crystalline polymers are characterized by a wide variety of morphological forms from dendrites to single crystals. In its turn, the capacity for crystallization is determined by features of the molecular structure of the polysaccharide.

If a polysaccharide consists of monotypical monoses, contains one and the same type of bonds between the monomeric units, and possesses a stereoregular structure, it crystallizes readily and has a tendency to form the most diverse supermolecular structures. Such substances are, for example, cellulose [10], chitin, and inulin [8], which give the x-ray patterns of highly crystalline substances with a large number of reflections and are capable of undergoing ordering in the form of crystals, lamellae, fibrils, etc. At the same time, if the chain of the polysaccharide contains residues of different monoses and different types of glycosidic bonds, as is the case, for example, of a glucofructan consisting of fructose and glucose units possessing (2 → 1)- and (2 → 6)-glycosidic bonds in a ratio of 2.7:1, the substantial disturbance of the regularity of the molecular structure leads to a loss of capacity for crystallizing.

Precisely the same thing is observed in the case of a glucomannan [7] containing in its chain glucose and mannose residues linked by β-(1 → 4)-glycosidic bonds. It is probable that, in this case, the possibility of the formation of a regular system of intermolecular hydrogen bonds, which appears in polysaccharides of stereoregular structure and cannot be realized in the case of polysaccharides with different types of disturbances of the molecular order is of great importance. (The same factor is apparently responsible for the far higher water solubility of the glucofructan than of inulin and, especially, cellulose.) As a result, the appearance of three-dimensional order proves to be impossible, and the globular structures that are typical for amorphous polymers are formed.

It is important that an infringement of any of the conditions of regularity at the molecular level unavoidably leads to the corresponding structural changes. Extremely interesting is the higher orderedness of completely acetylated polysaccharides than of the initial polysaccharides, the former giving lamellar supermolecular formations with two-dimensional order, as is shown by the x-ray results. This fact undoubtedly requires further investigation.

EXPERIMENTAL

Paper chromatography was performed on Filtrak FN-3,12 paper in the following solvent systems (amounts by volume): 1) butan-1-ol—pyridine—water (6:4:3); 2) phenol—butan-1-ol—acetic acid—water (5:5:2:10). TLC was performed on Silufol UV-254 plates and on plates coated with KSK silica gel in the following systems: 3) methyl ethyl ketone—1% NH_3 (30:1); 4) benzene—acetone (2:1); and, 5) chloroform—methanol (9:1). To indicate the spots we used the following reagents: 1) aniline hydrogen phthalate (10 min at 105-110°C); 2) a 5% ethnolic solution of urea; 3) a 0.2% ethanolic solution of resorcinol; and, 4) a 1% solution of $KMnO_4$—benzidine. GLC was performed on a Tsvet-101 instrument with a flame-ionization detector and a stainless-steel column (0.3 × 200 cm) under the following conditions: A) 5% of silicone XE-60 on Chroamton NAW (0.200 × 0.250 mesh), 150°C; 60 mm/min He; and, B) on a Chrom-1 instrument with 20% of BDS, 60°C, 50 ml/min of He, for the methylated pseverin derivatives. The IR spectra of the samples were taken on a UR-20 instrument, using tablets with KBr.

The ^{13}C NMR spectrum was taken on a WM-60 instrument (Bruker) using a 3% solution in D_2O at 35°C with CH_3OH as internal standard, its signal being 50.15 ppm from that of tetramethylsilane.

The electron-microscope observations were made on 4 ÉVM-100 K and Tesla BS-242E microscopes. The samples for investigation were prepared by the suspension or the attachment method, after which they were shadowed with Pt/C in vacuum. The x-radiographic studies were performed on a DRON-2 instrument with monochromatized Cu K_α radiation. The samples were molded in the form of tablets. Recording conditions: voltage 20 kV; current strength 30 mA.

Isolation of Pseverin. After the precipitation of the WSPS with ethanol from the mother solution, the total glucofructan was obtained with a yield of 60.11 g [1]. A solution of 10 g of the glucofructan in 100 ml of water was treated with a 10% solution of $Pb(CH_3COO)_2$ and with a saturated solution of Na_2SO_4, and the resulting precipitate was centrifuged off. The supernatant was dialyzed against distilled water and the undialyzed part was evaporated to a syrup which, on being triturated in a mortar with acetone, gave a white powder of the glucofructan pseverin. Yield 0.75 g, $[\alpha]D^{22}$ − 30° (c 0.4; water).

Hydrolysis of Pseverin. A mixture of 0.1 g of pseverin and 3 ml of 0.5 N H_2SO_4 was boiled in the water bath for 2 h. The hydrolysate was neutralized with $BaCO_3$, deionized with KU-2 anion-exchange resin (H^+), evaporated to 1 ml, and investigated by PC (systems 1 and 2; revealing agents 1 and 2); fructose and glucose were detected. The fructose was isolated and identified in the form of the isopropylidene derivative (II).

The quantitative ratio of the monosaccharides in pseverin was determined by a standard method [3].

Periodate Oxidation of Pseverin. A solution of 0.0413 g of the glucofructan in 16.6 ml of water was treated with 3.4 ml of 0.25 M $NaIO_4$. Oxidation was carried out at +4°C for 91 h. The consumption of periodate and the yield of HCOOH were determined by the titrimetric method. Glycerol was detected in the product of Smith degradation by PC (system 1, revealing agent 4).

Methylation of Pseverin. The methylation of 0.15 g of the substance dissolved in DMSO was performed by Hakomori's method [4]. The yield of permethylate was 0.096 g, the completeness of methylation being determined by IR spectroscopy.

Methanolysis of Pseverin. A solution of 0.05 g of the permethylate in 2 ml of a 2% solution of HCl in methanol was kept at 60°C for 2 h. Then it was evaporated to dryness with methanol several times, and 2,3,4,6-tetra-O-methyl-D-glucose, 1,3,4,6-tetra-O-methyl-D-fructose, 1,3,4-tri-O-methyl-D-fructose, and 3,4,6-tri-O-methyl-D-fructose were detected by TLC (systems 3 and 4, revealing agents 1, 2, and 3) and by GLC (conditions B), in comparison with authentic samples [5].

CONCLUSIONS

1. The homogeneous glucofructan pseverin has been obtained by dialyzing the total glucofructans from the rhizomes of Polygonatum sewersowii Regel. It has been established by chemical and spectral methods that it has a linear structure with a chain containing 2 → 1- and 2 → 6-bound fructofuranose residues. This type of glucofructan has not hitherto been detected in the family Liliaceae.

2. A correlation has been found between the molecular structure of a glucofructan and its supermolecular organization. A disturbance of the regularity of the chemical structure in the chain leads to the amorphization of the polymer and to a unification of its morphological structure.

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