POLYSACCHARIDE COMPOSITION OF *Polygonum aviculare*

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A polysaccharide isolated from the epigeal parts of *Polygonum aviculare* has been fractionated. It has been shown that the initial polysaccharide consists of at least four fractions differing in their monosaccharide compositions and physicomechanical properties.

Carbohydrates of plants of the *Polygonaceae* family have been considered in [1-3]. The water-soluble polysaccharides have been studied inadequately. Continuing our investigation [4, 5], we have fractionated polysaccharides isolated from the epigeal parts of *Polygonum aviculare* collected in the environs of Ryazan' in the period of the maximum accumulation [5].

The air-dry raw material was boiled in ether to eliminate pigments and low-molecular-weight impurities. The plant residue after ethereal extraction was dried and was heated with water, and the water-soluble polysaccharides were precipitated with ethanol. The polysaccharides were freed from accompanying protein by Sevag's method [6] and were demineralized with the aid of the ion-exchange resins KU-2 (H^+) and AV-17 (OH^-). Alkaline saponification was performed [7] and polysaccharides (I) and (II) were obtained in a weight of 3:2 (preparatively). We achieved a similar separation by treating the initial polysaccharide by Fehling's method. The polysaccharides (I) and (II) isolated by this method differed insignificantly from those obtained by alkaline saponification.

When polysaccharide (I) was treated with sodium acetate [8], polysaccharide A was obtained which, according to PC and GLC, consisted of galacturonic acid and rhamnose residues. Its homogeneity was confirmed by chromatography on DEAE-cellulose. From the mother liquor, two volumes of ethanol precipitated polysaccharide B, consisting of galacturonic acid, galactose, and rhamnose residues.

For a strict identification of the uronic acid in the polysaccharide fraction (I), its solution was converted into the methyl ester by treatment with diazomethane and this was reduced with sodium tetrahydroborate [9], giving an almost neutral glycan from the hydrolysis products of which galactose and uronic and aldobiuronic acids were isolated. The galactose was separated by electrophoresis and was identified by oxidation with nitric acid to mucic acid with mp 212-214°C.

When polysaccharide (I) was subjected to enzymatic hydrolysis [10], rhamnose, arabinose, glucose, and galactose were detected by PC and GLC in a quantitative ratio of 1:2:6:37, respectively, and galacturonic acid was isolated in the crystalline state with mp 155-156°C.

On acid hydrolysis and subsequent separation by paper electrophoresis, three zones were obtained which, according to PC results, corresponded to neutral monosaccharides, aldobiuronic acids, and polysaccharides. The zone corresponding to the aldobiuronic acids was isolated, treated with diazomethane, and reduced with sodium tetrahydroborate, giving dulcitol and sorbitol, in a ratio of 7:1 according to GLC.

With the aid of Cetavlon [11], fraction (II) yielded two polysaccharides, C and D, differing in their monosaccharide compositions and physicochemical properties.

The characteristics of the fractions and the quantitative ratios of the monosaccharides in them (moles) are given below:

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Fraction	$[\alpha]_D$	*Gal UA*	*Gal*	*Glu*	*Ara*	*Ram*
Initial	+116	46	14	1	28	4
I	+214	75	1	10	1	10
II	+62	23	2	2	1	1
A	+269	92	—	—	—	+
B	+226	65	4	—	—	1
C	+73	+	2	—	1	9
D	+28	+	10	3	1	9

EXPERIMENTAL

The samples of polysaccharides were hydrolyzed with 2 N sulfuric acid solution in sealed tubes at 100°C for 8-h, followed by neutralization with barium carbonate and treatment with KU-2 cation-exchange resin (H^+). Solutions were evaporated in a rotary evaporator at 35-40°C. Optical activities were determined on a EPN-Al instrument, and uronic acids as described in a handbook [12]. FN-7 and FN-11 papers were used for descending chromatography in the following solvent system (by volume): 1) butan-1-ol—pyridine—water (6:4:3); 2) ethyl acetate—formic acid—water—acetic acid (18:1:4:3); 3) ethyl acetate—pyridine—water—acetic acid (5:5:3:1). For detecting reducing monosaccharides, the chromatograms were treated with Bonner's aniline phthalate reagent [13], and for polyols with a solution of potassium permanganate—sodium periodate [14]. The GLC of the samples was performed on a Tsvet-4-67 instrument with a flame-ionization detector and a glass column (150 × 0.3 cm). Conditions: A) 5% of XE-60 on Chromaton N-AW-DMCS 0.16-0.2 mm, 210°C, air, 300 ml/min, hydrogen and helium 60 ml/min each for the aldonitrile acetates [15]; B) 3% of poly(neopentyl glycol adipate) under the same conditions for the polyol acetates [16]. Electrophoresis was performed in phosphate buffer with pH 8.7 at a current strength of 40 mA and a voltage of 200-400 V for 0.5-2 h.

The isolation and purification of the polysaccharides obtained from the epigeal parts was carried out as described previously [5].

Alkaline Saponification. A solution of 20 g of the initial demineralized polysaccharide in 770 ml of water was treated with 30 ml of a 1 N solution of sodium hydroxide. The resulting mixture was kept at room temperature for 2 h with continuous stirring. Then the alkali was neutralized with an equivalent amount of a 1% solution of hydrochloric acid. The precipitate that then deposited (fraction I) was washed with dilute hydrochloric acid, with ethanol, and with acetone, and was dried in vacuum. The supernatant liquid was evaporated in a rotary evaporator and was precipitated with a double volume of ethanol (fraction II).

Methylation. The polysaccharide (I) (2 g) was triturated in a 1:2 mixture of methanol and water, and then 100 ml of an ethereal solution of diazomethane was added and the mixture was kept at +5°C for 8 h. The product was filtered off, washed with ether, and dried. Yield 1.6 g.

Reduction. A solution of 0.25 g of sodium tetrahydroborate in 20 ml of water with the addition of glycerol was poured into 50 ml of water containing 1.6 g of esterified acid, and the mixture was placed in the refrigerator for 12 h. Then it was neutralized with acetic acid, dialyzed, treated with cation-exchange resin, and evaporated three times with methanol. Methylation and reduction were repeated five times. The yield of glycan was 1 g.

Isolation of D-Galactose. The glycan (1 g) was hydrolyzed with a 1 N solution of sulfuric acid for 5 h. The hydrolysate was neutralized with barium carbonate and was evaporated to small volume, after which the barium salts of the acidic sugars were precipitated with ethanol. The precipitate was separated off by centrifugation. The filtrate was evaporated and the residue was crystallized from ethanol. D-Galactose was isolated with $[\alpha]_D$ + 83° (c 0.1; water); mp 163-164°C.

Fractionation with Sodium Acetate. A homogeneous solution of 8 g of fraction (I) in 600 ml of water containing 12 ml of caustic soda was stirred while 170 ml of 2 N sodium acetate was added. The resulting precipitate was separated off by centrifugation and was dried by means of a change of solvents. Yield 3.1 g (polysaccharide A). The solution was dialyzed for three days, concentrated, and poured into six volumes of ethanol, and the precipitate was separated off. Yield 2.6 g (polysaccharide B).

Chromatography on DEAE-Cellulose. DEAE-cellulose (40 g) was treated three times with 0.5 N hydrochloric acid solution and three times with 0.1 N sodium hydroxide solution and was then placed in a 40 × 4-cm chromatographic column and was washed with 2 liters of phosphate

buffer having pH 5.86. The excess of phosphate was eliminated. Then 0.4 of the polysaccharide was deposited on the column and was eluted with 1 liter of buffer solution and then with increasing concentrations of sodium hydroxide of from 0.01 to 0.1 N using 0.5 liter of 0.01 N and 1 liter of 0.1 N solutions. Fractions with a volume of 30 ml each were taken off and the presence of the polysaccharide in aliquots was determined by the phenol/sulfuric acid method. The fractions corresponding to a single peak were combined and dialyzed, and the polysaccharide was isolated as described above. Yield 0.32 g.

Precipitation with Fehling's Solution. A solution of 2 g of the polysaccharide (fraction I) in 200 ml of water was treated with 18 ml of Fehling's solution. After 4 h, the precipitate was filtered off and was washed with acidified ethanol and then with acetone until copper chloride was no longer present, after which it was dried. Yield 1.7 g.

Enzymatic Hydrolysis. With heating, 2 g of the polysaccharide (fraction A) was dissolved in 60 ml of water and the pH of the solution was brought to 3.5 with 2 N caustic soda solution, after which 0.2 g of pectinase and three drops of toluene were added. Hydrolysis was performed in a thermostat at 38-40°C. The degree of hydrolysis was followed by the PC method. After eight days the solution was neutralized with 1 N sulfuric acid solution and was treated with KU-2 cation-exchange resin (H^+) and evaporated. The syrup was poured into a mixture of diethyl ether and ethanol (3:5) and the mixture was left at +5°C for 12 h. The resulting ethereal-alcoholic solution was concentrated, and crystals of D-galacturonic acid were isolated with $[\alpha]_D$ +54.8° (c 0.1; water).

Fractionation with Cetavlon. A solution of 2 g of the polysaccharide (fraction II) in 200 ml of water was neutralized with 0.5 N ammonia solution to pH 7, and 75 ml of a 3% solution of Cetavlon was added with gentle heating (37°C), after which the precipitate that deposited was separated off by centrifugation. It washed with water and was dissolved in 60 ml of a 10% solution of sodium chloride. The homogeneous solution was treated with 35 ml of a 5% solution of potassium iodide, the resulting precipitate of Cetavlon was discarded, and the solution was washed twice with chloroform. The aqueous solution was treated with cation-exchange resin, evaporated, and precipitated with ethanol, and the precipitate was dried by means of a change of solvents. This gave polysaccharide C with a yield of 0.7 g.

The solution was treated similarly with a 5% solution of potassium iodide until the turbidity had disappeared, the precipitate was removed, and the solution was concentrated, to give polysaccharide D. Yield 0.8 g. The monosaccharide compositions of polysaccharides C and D were investigated by PC and GLC.

SUMMARY

1. A water-soluble complex has been isolated from a medicinal raw material, the epigeal part of *Polygonum aviculare*, and its mono- and polysaccharide compositions have been studied.

2. It has been established that the polysaccharide complex is not homogeneous but consists of at least four polysaccharides differing in their monosaccharide compositions and physicochemical properties.

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SOME ASPECTS OF THE STEREOCHEMISTRY AND NOMENCLATURE OF POLYUNSATURATED HYDROXY FATTY ACIDS

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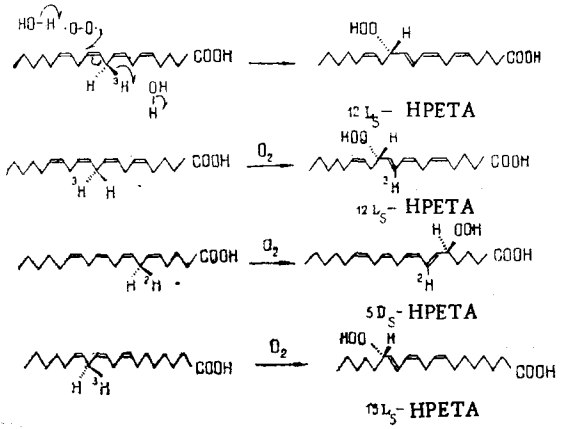
When the D, L (but not the R, S) nomenclature is used, general laws are observed in the stereochemistry of lipoxygenase oxidation reactions and in the formation of epoxides from hydroperoxides of polysaturated hydroxy fatty acids. In both cases the formation of a C—O bond is coupled with the stereoselective elimination of a hydrogen atom, and the chiral and prochiral carbon atoms have identical configurations. The use of the D, L nomenclature in the polyenic hydroxy fatty acid series appears preferable to that of the R, S nomenclature.

In a study of the lipoxygenase oxidation of unsaturated fatty acids, a rule has been found which relates to the stereochemistry of the inclusion of an oxygen atom and the elimination of hydrogen [1-7]. The formation of hydroxy acids with the L configuration of the asymmetric center is accompanied by the stereoselective elimination of a tritium atom present in prochiral centers with the pro-L-configurations in the molecules of radioactively labeled fatty acids [1-4, 6, 7].

Analogously, the lipoxygenase oxidation of $7L_R$-^3H (or ^2H)-arachidonic acid leads to the formation of 5-D_S-HPETA quantitatively containing the whole of the isotopic label, which indicates a stereoselective elimination of the 7D_S hydrogen atom [5-7]. The inclusion of an oxygen atom and the elimination of a hydrogen atom take place in opposite directions from the plane in which the carbon atoms of the penta-cis-1, cis-4-diene grouping are located with a fixed conformation of the C—C bonds, as shown in scheme 1*.



*Abbreviations: 12L_S-HPETA — (12S)-12-hydroperoxyeicosa-5Z, 8Z, 10E, 14Z-tetraenoic acid; 15L_S-HPETA — (15S)-15-hydroperoxyeicosa-8Z, 11Z, 13E-trienoic acid; 5D_S-HPETA — (5S)-5-hydroperoxyeicosa-6E, 8Z, 11Z, 14Z-tetraenoic acid; 5D_S-HPETA — (5S)-5-hydroperoxyeicosa-6E, 8Z, 11Z, 14Z, 17Z-pentaenoic acid; 9L_R-HPODA — (9R)-hydroperoxyoctadeca-10E, 12Z-dienoic acid; 13L_S-HPODA — (13S)-13-hydroperoxyoctadeca-9Z-11E-dienoic acid.

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