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POLYSACCHARIDE FROM THE LEAVES OF *Phytolacca americana*

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A polysaccharide has been isolated from the leaves of *Phytolacca americana* and has been characterized. It has been established that it contains residues of galactose, arabinose, xylose, and rhamnose, in a ratio of 3:4:1:3 and also D-galacturonic acid (85-90%). The results obtained permit the polysaccharide to be assigned to the class of pectin substances.

Performing a systematic search for antitumoral substances among polysaccharides (PSs) isolated from higher plants, we have investigated representatives of the family *Phytolaccacea*. The results of the investigation have shown that definite interest in this direction is presented by a mixture of polysaccharides isolated from the plant *Phytolacca americana* L. After preliminary treatment with boiling 82% ethanol, hot water extracted a mixture of polysaccharides with a yield of 5% from the leaves of the plant. Chromatography on a column (2.9 × 30 cm) containing DEAE-cellulose with subsequent elution by water, 0.2 M Na phosphate buffer (pH 8.9), and 0.02 N and 0.2 N NaOH separated this mixture (300 mg) into three polysaccharide fractions. The first of them, eluted from the column with the phosphate buffer, was studied in more detail: its yield amounted to about 60% on the polysaccharide mixture.

Gel chromatography on Sephadexes G-100, G-150, and G-200, paper electrophoresis, and ultracentrifugation showed that the fraction isolated was an individual polysaccharide with $[\alpha]_D^{20}$ +228° (c 0.5; 0.02 N NaOH). The polysaccharide obtained was soluble in weakly alkaline solutions of salts, contained about 2% of $-OCH_3$ groups and, according to the results of paper electrophoresis, possessed acidic properties.

On acid hydrolysis (2 N H_2SO_4, 100°C, 8 h) the products were found by paper chromatography (PC) in systems 1 and 2 to contain galactose, arabinose, xylose, and rhamnose in a ratio of 3:4:1:3, and a uronic acid. These results were confirmed in parallel by the GLC method. The amount of uronic acid in the polysaccharide, determined by the carbazole method [1] was 85-90%.

For a strict identification of the nature of the uronic acid, the polysaccharide was subjected to stepwise hydrolysis successively with 30% HCOOH (100°C, 1 h) and 1 N H_2SO_4 (100°C, 18 h). The main component found in the hydrolysate after the treatment with 1 N H_2SO_4 was a uronic acid having a R_f value identical with that of the galacturonic acid taken as a marker. By its sorption on AV-17 anion-exchange resin (HCO_3^-) from the hydrolysate of the polysaccharide and its desorption with 10% acetic acid, the uronic acid was isolated in the individual state. Its oxidation with 25% nitric acid led to the formation of mucic acid,

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which was identified by a mixed melting point with an authentic sample (208-210°C). An indirect confirmation of the nature of the uronic acid was also its disappearance from a hydrolysate of the polysaccharide that had been esterified and reduced with sodium tetrahydroborate [2] and an increase in the amount of galactose in it as compared with the amount formed in the hydrolysis of the initial polysaccharide.

Additional information on the structure of the polysaccharide was obtained by studying the products and enzymatic hydrolysis. Heating the polysaccharide at 100°C with Dowex 50 × 10 times (H^+ form) or with 30% HCOOH led mainly to the splitting out of arabinose. After enzymatic hydrolysis of the polysaccharide with pectinase (37°C, 120 h), galacturonic acid and two oligosaccharides were isolated by preparative PC. Acid hydrolysis (0.05 N H_2SO_4, 100°C, 3 h) showed that the oligosaccharide having the greater mobility with R_{GalUA} 1.9 consisted of xylose residues (systems 2 and 3) and the oligosaccharide with the lower mobility of R_{GalUA} 1.5 consisted of galactose, arabinose, and galacturonic acid residues (systems 1 and 2).

In a hydrolysate after the periodate oxidation of the polysaccharide (0.3 M $NaIO_4$ in the dark, 72 h) followed by its Smith reduction, galactose, galacturonic acid, glycerol, and erythritol were found by paper chromatography.

Characteristic for the IR spectrum of the polysaccharide were absorption bands in the regions of frequencies of 1735 cm^{-1} (stretching vibrations of carboxylic carbonyl groups), 1000-1150 cm^{-1} (stretching vibrations of pyranose ring), and 850 cm^{-1} (α-glycosidic bonds).

Thus, the results presented in this paper permit us to consider that the main chain of the polysaccharide which we isolated from the leaves of *Phytolacca americana* consists of residues of D-galacturonic acid in the pyranose form with galactose residues included in it; arabinose residues (or at least some of them) occupy peripheral positions with respect to this chain. In addition to the monosaccharides mentioned, the polysaccharide contains xylose and rhamnose residues. The presence in the products of Smith degradation of glycerol and erythritol, the high value of the specific rotation of the polysaccharide, and its absorption in the UV spectrum at 850 cm^{-1}, and also its decomposition by pectinase show that the monosaccharide residues of the main chain of the polysaccharide and, in particular, the D-galacturonic acid residues are linked by α-(1→4)glycosidic bonds. The presence among the products of Smith degradation of unoxidized monosaccharide residues may be the result of the fact that they are either points of branching in the polysaccharide or are substituted at the C_3 hydroxyl in it.

EXPERIMENTAL

For descending paper chromatography we used Filtrak-11 paper (GDR) and the following systems of solvents: 1) ethyl acetate–acetic acid–formic acid–water (18:9:4:1); 2) butan-1-ol–benzene–pyridine–water (5:1:3:3); and 3) butan-1-ol–acetic acid–water (4:1:5), upper layer. The revealing agent was aniline hydrogen phthalate in water-saturated butanol.

The gas–liquid chromatography (GLC) of the trimethylsilyl derivatives of the monosaccharides was performed on Khrom-31 instrument with a thermal condictivity detector; steel column (0.3 × 200 cm), temperature 170°C, stationary phase 5% of OV-17 on Chromosorb, W, AW-DMCS; rate of flow of helium 20 ml/min.

Electrophoresis was performed on FN-1 paper (GDR) in 0.025 M pyridine-acetate buffer, pH 4.5, 0.2 M phosphate buffer pH 7.2, and borate buffer pH 12 (30 V/cm). The polysaccharide was detected with an alkaline solution of silver nitrate.

Gel chromatography on the Sephadexes was performed in 0.2 m phosphate buffer, pH 8.0. The solutions of the polysaccharide were evaporated in vacuum at 42°C.

The IR spectrum was recorded in tablets with KBr on a UR-10 instument (GDR).

The optical rotation of the PS was determined on an automatic polarimeter.

The hydrolysis of the polysaccharide with pectinase (Fluka) was performed by the method described by Yakovlev and Gorin [3], and periodate oxidation and Smith degradation by the procedure described by Dudkin and Ozolina [4].

The preparation and identification of the uronic acid from the polysaccharide was carried out either by converting it into galactose in the polysaccharide [2] degraded by 30% HCOOH or by isolating it with the aid of AV-17 ion-exchange resin (HCO_3^-) from an acid hydrolysate and its conversion into mucic acid.

Isolation of the Polysaccharide. After treatment with boiling 82% ethanol (1 h), the leaves were dried in the air and the polysaccharide mixture was extracted from them with water (65°C, 1 h). After dialysis against mains water and distilled water (7 days) and evaporation in a rotary evaporator, the polysaccharide mixture was precipitated with ethanol (1:4) and was freeze-dried. Then the individual polysaccharide was isolated from the mixture with the aid of DEAE-cellulose.

CONCLUSION

A polysaccharide has been isolated from the leaves of *Phytolacca americana* and has been characterized. It has been established that it contains residues of galactose, arabinose, xylose, and rhamnose in a ratio of 3:4:1:3 together with 85-90% of D-galacturonic acid residues. The results obtained permit the polysaccharide to be assigned to the class of pectin substances.

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NEW NATURAL PHENOLIC TRIGLYCERIDES

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The composition of the phenolic triglycerides isolated from ethanolic extracts of propolis, aspen buds, and wheat roots has been investigated. Two new phenolic triglycerides have been identified: 2-acetyl-1,3-diferuloylglycerol, and 2-acetyl-3-p-coumaroyl-1-feruloylglycerol.

Recently, new natural triglycerides substituted in positions 1 and 3 by residues of cinnamic acid and its derivatives were isolated from the buds of *Populus lasiocarpa* [1, 2]. Later, glycerides of this type were also found in the fruit of *Aegilops ovata* L. [3], and in these the hydroxyl group in position 2 remained unsubstituted.

We have also determined a fraction containing phenolic glycerides in ethanolic extracts of the buds of another species of poplar, *Populus tremula* L. (aspen), and also in the propolis which honeybees collect from the secretions of its axillary buds [4]. Since phenolic triglycerides are present in the buds themselves in extremely minute amounts, we used the more accessible propolis of the appropriate type. The triglyceride fraction of this product was isolated by chromatographing the dry residue of an ethanolic extract on columns of silica gel in a n-heptane–ethyl acetate gradient system. The isolation was monitored by TLC on Silufol. The phenolic triglycerides had R_f 0.7 in the benzene–ethyl acetate–methanol (10:4:1) system and were readily detected from the nature of their fluorescence in UV light or from the pinkish color of the spots when the plates were sprayed with concentrated H_2SO_4. The subsequent purification of the triglyceride fraction from the phenols accompanying it was performed by acetylation with Ac_2O in pyridine. The product formed, after the usual working up, was separated on preparative plates coated with silica gel by two runs of the ethyl acetate–n-heptane (1:2) system.

Below, we give the results of a mass-spectrometric analysis of the acetylated friction of the phenolic triglycerides from propolis:

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