CHEMICAL INVESTIGATION OF THE POLYSACCHARIDES OF THE LEAVES OF PLANTAGO MAJOR L.

II. Pectic Acid*

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In the chemical study of the pectins, it is of fundamental importance to establish the composition and properties of pectic acid, which is generally accompanied by galactoaraban and other polysaccharides as impurities [1], while in some cases it is not clear whether the neutral polysaccharides are only physical impurities or whether a chemical bond arises between them and the polyuronides.

Table 1

Composition and Properties of Plantain Leaf Polyuromides Obtained by Various Methods

Polysaccharides	$[\alpha]_D$, degrees	Amount of galacturonic anhydride, %	Constituents*								
			A	B	C	D	E	F	G	H	I
Initial polysaccharide, extracted with hot water	+195	68	+	+	+	+	+	+	+	+	+
Polysaccharide extracted with a 0.25% solution of a mixture of ammonium oxalate and oxalic acid	+207	71.5	+	+	+	+	+	+	+	+	+
Pectic acid of the polysaccharide after saponification	+242	83.1	+	+	+	+	−	±	−	+	+
Pectic acid after reprecipitation via calcium pectate	+242	82.5	+	+	+	+	−	−	−	+	+
Pectic acid isolated via cetyltrimethylammonium pectate	+230	78.6	+	+	+	+	−	−	−	+	+
Pectic acid obtained with the aid of DEAE-cellulose	+238	79.8	+	+	+	+	−	−	−	+	+

*A) galacturonic acid; B) galactose; C) rabinose; D) rhamnose; E) glucose; F) xylose; G) substance (VI); H) substance (VIII); I) substance (IX).

This paper gives the results of an investigation of the pectic acid contained in the leaves of Plantago major L. (the rippleseed plantain). To isolate this acid, we first used selective extraction of the pectic acid with a mixture of equal volumes of 0.25% solutions of ammonium oxalate and oxalic acid [2]. However, the polysaccharide isolated scarcely differed from the polysaccharide obtained by extraction with hot water [3]. Consequently, the subsequent fractionation experiments were carried out by using a polysaccharide isolated by the method developed previously [3]. When the polysaccharide was saponified with alkali [4] and the product subsequently purified, a pectic acid with a higher content of galacturonic anhydride than the initial polysaccharide was obtained (Table 1).

Reprecipitation of the pectic acid via calcium pectate and isolation of the polyuronide from the initial polysaccharide via cetyltrimethylammonium pectate [5] led to products with similar properties (Table 1). The polygalactiuronides obtained by the three methods contained galacturonic acid (78-83%), galactose, arabinose, rhamnose, and substances (VIII) and (IX). The neutral monosaccharides were apparently linked chemically with the main polygalacturonide chain. This assumption was confirmed by the fractionation of the initial polysaccharide on a column of DEAE-cellulose [6] (Table 2). In order to enhance the interaction of the polyuronide fraction of the polysaccharide with the anion exchanger, the initial polysaccharide was first treated with alkali to saponify the methyl ester groups of the pectic acid.

As can be seen from Tables 1 and 2, the polysaccharide of the leaves of the rippleseed plantain consists mainly of pectic acid and contains only a small amount of free galactoraban and galactan.

Experimental

The paper chromatography was carried out in the following systems: 1) butan-1-ol-pyridine-water (6 : 4: 3), 2) ethyl acetate–formic acid–acetic acid–water (48: 1: 3: 4).

*We have used Kertesz's nomenclature of pectic compounds [4].

Table 2

Composition of the Fractions of the Plantain Polysaccharide by Separation on DEAE-Cellulose

Fraction	Amount of eluate, ml	Weight of polysaccharides, mg	Monosaccharide composition of the fractions*								
			A	B	C	D	E	F	G	H	I
1	210	12	—	+	+	—	—	—	—	—	—
2	90	2	—	+	—	—	—	—	—	—	—
3	60	Traces	—	—	—	—	—	±	—	—	—
4	300	11	—	+	—	—	—	—	—	—	—
5	180	1.5	—	+	—	—	—	—	—	—	—
6	270	1.0	±	—	—	—	—	—	—	—	—
7	270	2.5	+	—	+	—	—	—	—	—	—
8	330	201	+	+	+	+	—	—	—	+	+
9	330	5.0	+	+	±	±	—	—	—	—	—
10	300	0.5	—	—	—	—	—	—	—	—	—

*Sugar symbols as for Table 1.

The amount of galacturonic anhydride was determined by decarboxylation in Anderson's apparatus [8]. The polysaccharides investigated were dried under vacuum over phosphorus pentoxide at a residual pressure of 0.03 mm Hg at 80°C for 10 hr.

The polysaccharide was fractionated on diethylaminoethylcellulose (DEAE-cellulose) with a capacity of 0.52 meq per g [7].

Selective extraction of pectic acid. The extractable substances were removed from 100 g of the leaves with 80% ethanol [1, 3] and the pectic acid was extracted with a mixture of equal volumes of 0.25% solutions of ammonium oxalate and oxalic acid (1: 20) at 80°C [2]. The polysaccharide was precipitated with alcohol, dissolved in water, and treated with KU-2 (H^+) to eliminate cations. Then the pectic acid was precipitated with three volumes of alcohol and was washed with 80% and 90% ethanol and with acetone. This gave 2.5 g of polysaccharide.

Preparation of pectic acid after saponification of the polysaccharide with alkali. A solution of 10 g of the initial polysaccharide (see Table 1) in 200 ml of water was treated with 50 ml of a 1 N caustic soda solution. After saponification for 30 minutes at 18-20°C, an excess of a 1 N solution of hydrochloric acid was added to the solution at 18-20°C. The precipitate of pectic acid was centrifuged off and then dispersed in 1 *l* of distilled water and washed free from acid with water and alcohol. This yelded 4.9 g of pectic acid.

Purification of pectic acid via calcium pectate. 2 g of the pectic acid obtained in the preceding experiment was dispersed in 20 ml of water, and an equivalent amount of 0.5 N ammonia solution was added followed by water to 100 ml. This solution was then treated with a 5% solution of calcium chloride until no further precipitate of calcium pectate was deposited. The calcium pectate was separated off, washed with water to remove calcium chloride, and treated with a 1.5% solution of ammonium oxalate at 60°C to bring the pectic acid into solution and precipitate the calcium ions. The filtrate was dialyzed against distilled water for 3 days. Then the ammonium salt of pectic acid was precipitated with alcohol and washed with acetone. This gave 1.4 g of ammonium pectate, which was converted into the acid by acidification.

Isolation of pectic acid via cetyltrimethylammonium pectate. A solution of 2 g of the initial polysaccharide in water (20 ml) was neutralized with a 0.5 N solution of ammonia. Then the pectic acid was precipitated from a 2% solution with a 3% solution of cetyltrimethylammonium bromide (Cetavlon) in water. The precipitate of cetyltrimethylammonium pectate was centrifuged off, washed several times with water, and dissolved in 15 ml of a 10% solution of sodium chloride. The solution was treated with a 5% solution of potassium iodide and chloroform to eliminate the cetyltrimethylammonium ion. The cations were separated by treatment with KU-2 (H^+), and the polysaccharide was precipitated with alcohol. The yield of pectic acid was 0.8 g.

Fractionation of polysaccharide on a column of DEAE-cellulose.

A. Preparation of material for fractionation. 2 g of the initial polysaccharide was dissolved in 20 ml of water and saponified with a 1 N solution of caustic soda as described above. The polysaccharide was precipitated with four volumes of a 1% solution of hydrochloric acid in 95% ethanol and was washed with a 1% solution of hydrochloric acid in 80% ethanol to remove the inorganic salts. Then the polysaccharide was freed from acid with 80% alcohol and was washed with acetone and ether. This gave 1.4 g of polysaccharide containing 71.3% of galacturonic anhydride. All the other components of the initial polysaccharide were found in the hydrolyzate of the polysaccharide.

B. Fractionation of the polysaccharide. A dispersion of 0.250 g of the polysaccharide in 3 ml of water was neutralized with a 0.1 N solution of ammonia. The solution was transferred to a column of DEAE-cellulose in the phosphate form (pH 6; 220 × 25 mm). Elution was carried out by the gradient method with 0.5 N phosphate buffer (pH 6) by syphoning 800 ml of buffer into 500 ml of water. After 6 fractions had been collected (see Table 2), elution was continued under similar conditions with a 0.2 N solution of caustic soda. 30-ml portions of the eluate were analyzed for their polysaccharide content by the phenol method [9]. The eluates containing polysaccharides were treated successively with the ion-exchangers KU-2 (H^+) and Av-17 (OH^-) to eliminate mineral salts, and were then evaporated to dryness under vacuum at 45°. The residues were weighed and were hydrolyzed with 1 N sulfuric acid as described previously [1, 3]. The monosaccharide composition was determined by paper chromatography (see Table 2).

We used type "B" paper of the Leningrad mill for chromatography.

Summary

1. Pectic acid contains galacturonic acid (78-83%) and, chemically linked with it, the monosaccharides D-galactose, L-arabinose, L-rhamnose, and substance (VIII) and (IX).

2. By fractionating the polysaccharide complex of the leaves of Plantago major L. on a DEAE-cellulose column it has been shown that it consists of pectic acid (80-82%), and free galactoraban (5-6%) and galactan (4-5%).

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