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

I. Analysis of the Monosaccharide Composition of the Polysaccharide Complex

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The leaves of Plantago major L. (rippleseed plantain) are an accepted medicinal agent for gastroenteritic and other diseases [1]. The chemical composition and the active components of this plant have not been investigated adequately.

Until recently, only the polysaccharides in the seeds of a few species of plantain had been studied chemically [3]; the polysaccharide complex of rippleseed plantain leaves is now considered for the first time.

The basis for a detailed study of the polysaccharides of the rippleseed plantain leaves was a preparation consisting of the non-demineralized polysaccharide complex which, according to the pharmacological data of G. V. Obolentseva and Ya. I. Khadzhai, possesses marked anti-ulcer activity [2].

Table 1

No.	Monosaccharides	R (rhamnose)	Monosaccharide composition of hydrolyzates on stepwise hydrolysis of the polysaccharides				
			Time, hours				
			2	4	6	8	10–21
1	Galacturonic acid	0.01	+	+	+	+	+
2	Galactose	0.65	+	+	+	+	+
3	Glucose	0.71	+	—	—	—	—
4	Arabinose	0.77	+	+	—	—	—
5	Xylose	0.81	+	—	—	—	—
6	Substance (VI)	0.93	+	—	—	—	—
7	Substance (VII) (rhamnose)	1.00	+	+	+	—	—
8	Substance (VIII)	1.40	+	—	—	—	—
9	Substance (IX)	1.73	+	—	—	—	

Note; The R values for the sugars Nos. 1-7 were obtained using system A and those for substances Nos. 8 and 9 using system B.

This paper presents the results of an investigation into the monosaccharide composition of the polysaccharides in the leaves of the rippleseed plantain.

It was first established that the polysaccharide material contains up to 28% of nonignitable matter of which only up to one half is removed on prolonged dialysis.

The nondemineralized polysaccharide was hydrolyzed with 1 N sulfuric acid, and the products were analyzed by paper chromatography using several solvent systems. It was found that the polysaccharides contain nine monosaccharide components (Table 1). Judging from the chromatograms, the main components of the polysaccharide are galacturonic acid, galactose, arabinose, and rhamnose. Glucose and xylose were found in very small amounts. There are also weak spots formed by three unidentified monosaccharides – substances (VI), (VIII), and (IX).

The demineralized polysaccharide material was obtained by treating its aqueous solution with the cation-exchange resin (KU-2 (H^+)). The polysaccharide obtained in this way consisted of a greyish-white powder that had the properties of an organic acid (a 1% aqueous solution of the polysaccharide had pH ~ 3). From the results of a quantitative determination of galacturonic anhydride (66-68%) [4], and also those of the potentiometric titration of the polysaccharides before and after saponification [5], it can be seen that galacturonic acid is the main component of the polysaccharide complex in the plantain leaves.

In order to establish the quantitative ratio of the neutral sugars present in the polysaccharide and to find the optimum conditions for their isolation, we studied the hydrolysis of the polysaccharide to monosaccharides by separating incompletely hydrolyzed polysaccharide fractions after determined intervals of time. These fractions were purified and then hydrolyzed with acid; the hydrolyzates so obtained were analyzed by chromatography (cf. Table 1).

It follows from Table 1 that the hydrolysis of the neutral sugars of the polysaccharide is complete after 6-8 hr.

The products of partial hydrolysis treatment with 1 N sulfuric acid for 8 hr were found to contain galacturonic acid and oligouronides (Table 2).

Table 2

R values (galacturonic acid) of the oligouronides	Qualitative oligouronide composition of the polysaccharide hydrolyzates as a function of hydrolysis time				
	Time, hours				
	8	12	16	20	21
0.16	+	+	±	−	−
0.37	+	+	+	±	−
0.82	+	+	+	+	−
1.00	+	+	+	+	+

Note. The R values for the compounds investigated were obtained using system C.

The quantitative determination of the main neutral monosaccharides (rhamnose, arabinose, and galactose) in the polysaccharide hydrolyzate was carried out by the method of G. N. Zaitseva and T. G. Afanas'eva [6] with aniline phthalate reagent (Table 3).

To isolate and identify the individual monosaccharides, the demineralized complex of the polysaccharide was hydrolyzed with sulfuric acid and neutralized with barium carbonate, the barium salts of the galactouronides were precipitated, and the neutral hydrolysis products were separated on a cellulose powder column using butan-1-ol saturated with water as the eluting solvent [7]. The separation yielded eight individual monosaccharides; information on their physicochemical properties is given in Table 4.

The unidentified monosaccharides (VIII) and (IX) with R_f (tetramethylglucose) 0.42 and 0.52, fall in the region of the dimethyl ethers of the hexoses [8]. On demethylation with 48% hydrobromic acid, substance (IX) formed L-galactose [9]. The demethylation results enabled us to assume that substance (IX) is partially methylated galactose.

Thus, chemical investigation of the polysaccharides of rippleseed plantain leaves has shown that they contain nine monosaccharides with D-galacturonic acid as the main component.

Table 3

Sugar	Weight of sugars μg				Weight ratio
	Runs				
	1st	2nd	3rd	4th	
Rhamnose	41	38	45	40	1
Arabinose	81	80	89	82	2
Galactose	122	120	134	125	3

Experimental

The analysis of the sugars by paper chromatography was carried out in the following solvent systems: A) butan-1-ol–pyridine–water (6 : 4 : 3); B) butan-1-ol – ethanol –water (4 : 1 : 5); C) ethyl acetate –formic acid –water –acetic acid (18 : 1 : 4 : 3); D) butan-1-ol –acetic acid –water (4 : 1 : 5).

Isolation of the polysaccharide complex. The dry leaves of rippleseed plantain were extracted 6-7 times with boiling 80% ethanol (1 : 20). They were then washed with acetone and dried in the air. The polysaccharides were extracted with hot water (1 : 15) for 2 hours. The extract was filtered and was treated with 3 volumes of 96% ethanol. The precipitate of polysaccharides was separated off, washed with ethanol and dried. The yield of nondemineralized polysaccharide amounted to 10% of the weight of the dry leaves; the ash content of the polysaccharide was 28%.

Preliminary investigation of the products of the acid hydrolysis of the polysaccharide. A solution of 0.5 g of the nondemineralized polysaccharide in 25 ml of 1 N sulfuric acid was hydrolyzed in a sealed tube in a boiling water bath for 6 hours. The hydrolyzate was neutralized with barium carbonate, filtered, and evaporated in vacuum to 1 ml. The hydrolysis products were analyzed by paper chromatography using systems A, B, and D. The hydrolyzate was found to contain the following nine monosaccharides: galacturonic acid, galactose, glucose, rhamnose, arabinose, xylose, and three unidentified monosaccharides (cf. Table 1).

Preparation of the demineralized polysaccharide. A solution of 10 g of nondemineralized polysaccharide in 100 ml of distilled water was transferred to a column containing the cation exchanger KU-2 (H^+) (20 × 180 mm). The column was eluted with water, and the aqueous eluates were mixed with alcohol in a ratio of 1 : 4. The suspension that formed was centrifuged, and the residue was washed with 80% and 90% ethanol, acetone, and ether. The yield was 4.5 g. For analysis, the polysaccharide was dried under high vacuum at 80°. The polysaccharide had an ash content of 0.4%; $[\alpha]_D$ +195.0° (c 0.43; in 0.3 N sodium hydroxide solution). The amount of galacturonic anhydride in the polysaccharide, determined by decarboxylation in 19% hydrochloric acid, was 68%. The equivalent weight of the polysaccharide was determined by potentiometric titration with alkali. Before saponification, it was 454, and after saponification 263. By Zeisel's method [10], the content of methoxy groups was 4.2%.

Stepwise hydrolysis of the polysaccharide. 1. One gram of polysaccharide was dissolved in 50 ml of 1 N sulfuric acid and hydrolyzed in a sealed tube in a boiling water bath for 2 hours. The hydrolyzate was neutralized with barium carbonate and filtered; the filtrate was evaporated to 5 ml, the products of partial hydrolysis were precipitated with 25 ml of ethanol, and the precipitate was separated off. The filtrate was then evaporated to small bulk and used for

analysis of the sugars. The residue, consisting of products of the partial hydrolysis of the polysaccharide and barium salts of galacturonic acid, was precipitated three times with alcohol and used for re-hydrolysis. These operations were repeated after every 2-4 hours' hydrolysis for 24 hours. The results of the stepwise hydrolysis are given in Table 1.

2. Four 0.1-g samples of the polysaccharide were hydrolyzed as described above for 8, 12, 16, and 20 hours. The precipitates, consisting of the products of the partial hydrolysis of the polysaccharide and the barium salts of galacturonic acid, were separated and were reprecipitated with alcohol. The purified precipitates were then dissolved in 5 ml of water, treated with the cation-exchanger KU-2(H^+) and filtered. The filtrates were evaporated and the hydrolysis products were analyzed by paper chromatography using system B (see Table 2).

Table 4

Components	Content, %	Mp, °C	$[\alpha]_D$, deg.	Sugar derivatives	Mp of the derivatives, °C
Galacturonic acid (I)	66.00	149–150	+50.0	Mucic acid	220—221
Galactose (II)	12.48	164—165	+83.0	Mucic acid	220—221
Glucose (III)	Trace	Syrup	—	—	—
Arabinose (IV)	8.32	157—159	+104.0	Osotriazole	80—81
Xylose (V)	0.10	Syrup	—	—	—
Substance (VI)	Trace	"	—	—	—
Rhamnose (VII)	4.16	90—92	+8.1	Osazone	180—182
Substance (VIII)	0.20	Syrup	—	—	—
Substance (IX)	0.25	"	—	Galactose	—

Quantitative determination of rhamnose, arabinose, and galactose in the total neutral sugars of the polysaccharide hydrolyzate. 0.1 g of the polysaccharide was dissolved in 5 ml of 1 N sulfuric acid and hydrolyzed for 8 hours as described above. The hydrolyzate was neutralized with barium carbonate and was filtered. The filtrate was evaporated to 1 ml and diluted with 10 ml 96% ethanol. The precipitate of oligouronides which formed was filtered off and washed with 80% alcohol, and twice reprecipitated from the aqueous solution with alcohol. The alcoholic-aqueous filtrates were combined and evaporated to dryness in vacuum. The dry residue, consisting mainly of the total neutral monosaccharides, was dissolved in water, and the solution was transferred to a 5-ml measuring flask and brought up to the mark with water. This solution was used for the quantitative determination of galactose, arabinose, and rhamnose. The monosaccharides were separated using system A (see Table 3).

Table 5

Fraction	Volume of eluate, ml	Residue, mg	Qualitative composition of the fraction
3—6	400	25	Substance (IX)
7—8	200	20	Substance (VIII)
9—12	400	150	Rhamnose
13—14	200	—	Mixture of rhamnose and substance (VI)
15—18	400	5	Substance (VI)
19—20	200	—	Mixture of substance (VI) and xylose
21—22	200	8	Xylose
23—24	200	—	Mixture of xylose and arabinose
25—32	800	300	Arabinose
33—34	200	—	Mixture of arabinose and glucose
35	100	—	Glucose
36—37	200	—	Mixture of glucose and galactose
38—57	2000	350	Galactose

Isolation of the monosaccharides. Ten grams of the demineralized polysaccharide was hydrolyzed with 1 N sulfuric acid for 8 hours as described above. The barium salts of galacturonic acid and of the oligouronides were used for the isolation of the galacturonic acids, and the total neutral monosaccharides (2.5 g) were separated on a cellulose powder column.

Isolation of galacturonic acid. The barium salts of the galacturonides (5 g) were hydrolyzed with 1 N sulfuric acid (1 : 50) in a boiling water bath for 14 hours. The hydrolyzate was neutralized with barium carbonate and filtered, and the filtrate was evaporated to 25 ml. The barium salt of galacturonic acid present in the filtrate was precipitated with alcohol, twice reprecipitated from aqueous solutions and dried. The residue (2 g) was dissolved in 50 ml of water, and

the solution was passed through a column of the cation-exchanger KU-2 (H^+). The eluates were evaporated to dryness and dissolved in 4 ml of alcohol. The galacturonic acid which separated from the alcoholic solution was recrystallized from water (see Table 4). Oxidation of this substance with concentrated nitric acid gave mucic acid with mp 220-221°.

Separation of the neutral sugars. The mixture of neutral sugars (2.5 g) was dissolved in 5 ml of water and transferred to a cellulose powder column (35 × 750 mm). The sugars were separated by the elution of the column with water-saturated butanol. The eluates were collected in 100-ml fractions and evaporated to dryness, and the monosaccharides present in them were determined qualitatively by paper chromatography using system A. The sugars were detected by aniline phthalate reagent (Table 5). The fractions containing mixtures of sugars were subjected to re-separation under similar conditions. Information on the physicochemical properties of the monosaccharides isolated and their derivatives is given in Table 4.

Demethylation of substance (IX). A solution of 10 mg of substance (IX) in 0.5 ml of 49% hydrobromic acid was heated on a boiling water bath for 5 min. The reaction mixture was poured into 10 ml of water and was neutralized with Amberlite IR-411 (OH^-). The filtrate was evaporated to small bulk and the products were analyzed by paper chromatography using systems A, B, and C. Galactose was found among the demethylation products.

For the chromatography we used Leningrad paper of grade "M".

Summary

1. A polysaccharide has been isolated from the leaves of Plantago major L. and its monosaccharide composition has been studied.

2. It has been established that the polysaccharide investigated contains D-galacturonic acid, D-galactose, D-glucose, D-xylose, L-rhamnose, L-arabinose, a partially methylated galactose, and two unidentified monosaccharides.

3. The high content of galacturonic residues (68%) in the polysaccharide enables it to be classified as a pectin.

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