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PLANT POLYSACCHARIDES

VIII. POLYSACCHARIDES OF *Lagochilus zeravschanicus*

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Water-soluble polysaccharides, pectin substances, and hemicelluloses have been isolated from the epigeal part of Lagochilus zeravschanicus *and characterized. The pectins isolated are characterized by a high degree of esterification of the carboxy groups, and their base consists of a fragment constructed from α-1→4-linked* D-*galacturonic acid residues.*

Representatives of the genus *Lagochilus* are a source of iridoid glycosides [1], and a tincture and extract of *Lagochilus* are used in medical practice as hemostatic agents [2, 3]. Earlier, in studying the carbohydrates of *Lagochilus usunachmaticus* we established that they possess anticoagulant activity [4]. In order to expand the variety of medicinal plants, we have investigated the epigeal part of *L. zeravschanicus* for its carbohydrate content.

Lagochilus zeravschanicus Bge (fam. Labiatae) is a perennial herbaceous plant growing on Uzbekistan territory in the Tashkent and Samarkand oblasts [5].

To eliminate pigmentary and low-molecular-mass compounds the air-dry raw material was extracted with chloroform and then with methanol. By extracting the residual raw material successively with water and then with a mixture of 0.5% solutions of oxalic acid and ammonium oxalate with heating we obtained, respectively, water-soluble polysaccharides (WSPSs) and pectin substances (PcSs). Hemicelluloses (HCs) were obtained by extraction with 10% NaOH.

The yields of the above-mentioned carbohydrates and also their qualitative monosaccharide compositions are given in Table 1. According to PC results, in addition to the sugars shown in Table 1, the WSPSs isolated also contained galacturonic acid. To obtain a neutral polysaccharide (PS), the WSPSs were passed through a column with DEAE-cellulose (CH_3COO^-), which gave a 10% yield of a PS consisting of galactose, glucose, and arabinose residues. Elution with sodium acetate (0.5 N) and caustic soda (0.1 N) led to the isolation of a fraction of polysaccharides of acid nature containing galacturonic acid together with glucose and galactose.

Thus, the WSPSs consisted of a mixture of acid and neutral polysaccharides.

The pectin substances formed a cream-colored powder readily soluble in water and alkali, with $[\alpha]_D$ +160° (*c* 0.2; 0.1 N NaOH), η_{rel} = 4.03 (*c* 1.0; water). The molecular mass of the pectin, found by the sedimentation method, was 65,000 [6]. Its qualitative characteristics, found by the titrimetric method, were as follows (%): free carboxy groups, K_f, 3.24; methoxylated carboxy groups, K_e, 9.0 [7]. The degree of esterification is then 73.5% and the amount of $O-CH_3$ groups 5.4%. All this permits the pectin under study to be assigned to the high-methoxyl group [8].

The IR spectrum of the pectin, showing absorption bands in the region of 1735 cm^{-1} (stretching vibrations of the carbonyls of ester groups), 1000-1150 cm^{-1} (stretching vibrations of a pyranose ring), and 850 cm^{-1} (α-glycosidic bonds), was characteristic for the IR spectra of the pectins of higher plants [9].

Partial acid hydrolysis gave the main polyuronide fragment of the pectin chain — a galacturonan.

During the first hour of hydrolysis, arabinose and galactose were split out, and in the second hour glucose and galacturonic acid, as well, appeared in the hydrolysate. The third hour was characterized by the presence in the hydrolysate of galactose, glucose, arabinose, and galacturonic acid and the formation of a galacturonan consisting only of galacturonic acid residues (Table 2).

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TABLE 1. Characteristics of the Carbohydrates of *L. zeravschanicus*

Carbohydrate	Yield, %	MM	Neutral monosaccharides		
			Gal	Glc	Ara
WSPSs	3.0	55000	3	2	1
PcSs	3.8	65000	1	2.5	4
HCs	2.7		1	5.8	3.1

TABLE 2. Partial Hydrolysis of the Pectin

Time of hydrolysis, h	Yield of galacturonan, %	$[\alpha]_D$, deg (*c* 0.5; H_2O)	MM	Monosaccharide composition			
				Gal	Glc	Ara	GalUA
1	80	+160	50000	+	+	–	+
2	58	+150	41000	+	–	–	+
3	20	+130	15000	–	–	–	+

The galacturonans formed a white water-soluble powder having a positive specific rotation and characterized by a molecular mass considerably lower than that of the initial pectin.

The *D*-galacturonic acid residues were present in the galacturonan in the pyranose form, as was confirmed by its IR spectrum, which had the same absorption bands as the pectin itself. The action of pectinase served as an additional proof of the presence of α-1→4-bonds between the *D*-galacturonic acid residues both in the initial pectin and in the galacturonan. The products of the enzymolysis of the pectin included neutral monosaccharides and galacturonic acid, and those of the galacturonan only galacturonic acid.

The hemicelluloses isolated consisted of a dark-colored powder readily soluble in water and alkali. By their monosaccharide composition they belonged to the arabinoglucans. A positive reaction with iodine showed the presence of starch.

Thus, various biopolymers are produced in the epigeal part of *L. zeravschanicus*: WSPSs consisting of acid and neutral polysaccharides, PcSs similar to the pectins of higher plants, and hemicelluloses belonging to the class of arabinoglucans.

EXPERIMENTAL

Descending paper chromatography (PC) was conducted with Filtrak FN-12,13 paper. Solvent system: butan-1-ol–pyridine–water (6:4:3); revealing agent: acid aniline phthalate (100-105°C).

GLC was conducted on a Chrom-5 chromatograph with a flame-ionization detector; steel column (0.3 × 200 cm) filled with 5% of XE-60 on Chromaton N-AW 0.200-0.250 mm; carrier gas: helium (60 ml/min), 210°C.

Samples were hydrolyzed with 2 N H_2SO_4 at 100°C — WSPSs for 10 h and PcSs and HCs for 20 h. Derivatives for GLC — aldononitrile acetates — were obtained as described in [10].

Ultracentrifugation was carried out on a MOM-3170 instrument (50,000 rpm) at 20°C with an exposure rate of 30 min, using 1% aqueous solutions of the polysaccharides.

IR spectra of the samples were taken on a Perkin-Elmer IR Fourier spectrometer, model 2000, in tablets molded with KBr.

Viscosities were measured by an Ostwald viscometer with a diameter of 0.73 mm.

Galacturonic acid contents were determined by the colorimetric carbazole method [11].

Specific rotations were measured on a Zeiss polarimeter in a tube 1 dm long at 20°C.

Inactivation of the Raw Material. With boiling in the water bath, 100 g of the comminuted raw material (epigeal part) was treated successively with chloroform (1:5) and methanol (1:7). Then the residual raw material was filtered off and dried, and the WSPS, PcSs, and HCs were extracted.

Isolation of the WSPSs. The raw material was extracted with water twice for 2 h each time. The extracts were dried and evaporated, and the WSPSs were precipitated with methanol (1:10). The deposit of WSPSs was filtered off, washed with methanol, and dried. Yield, 9 g.

Isolation of the PcSs. The residual raw material was treated with a mixture of equal volumes of 0.5% solutions of oxalic acid and ammonium oxalate (1:10) at 80°C for 2 h twice. The extracts were evaporated and dialyzed against distilled water, and the PcSs were precipitated with methanol (1:10), washed on the filter with methanol, and dried. Yield of PcSs, 3.8 g.

Isolation of the HCs. The raw material after the isolation of the PcSs was extracted with 10% NaOH solution (1:10) at room temperature for 2.5 h. After filtration, the extract was neutralized with acetic acid and dialyzed. The dialysate was

evaporated and precipitated with methanol (1:10), and the precipitate was filtered off, washed with methanol, and dried. Yield of HCs, 2.7 g.

Separation of the WSPSs on DEAE-Cellulose. A solution of 1 g of WSPSs in 10 ml of water was deposited on a column of DEAE-cellulose (acetate form, 3 × 20 cm). After elution with water (500 ml) the eluates were evaporated and precipitated with methanol, and the precipitate was separated off and dried. The yield of neutral polysaccharide was 0.2 g. Then the column was washed with a 0.5 N solution of sodium acetate (500 ml) and, after that, with a 0.1 N solution of caustic soda (500 ml). The eluates were evaporated and precipitated with methanol. Yields of acid polysaccharides were 0.21 and 0.1 g.

Partial Hydrolysis of the PcSs. The PcSs (2 g) were dissolved in 300 ml of 0.5 N H_2SO_4 and hydrolyzed at 100°C for 1 h. The reaction mixture was dialyzed to a negative reaction for SO_4^{-2}. The dialyzed solution was evaporated and precipitated with methanol (1:10). The precipitate was centrifuged off and washed with methanol. This gave 1.6 g of partially hydrolyzed pectin (galacturonan). The galacturonan was hydrolyzed and analyzed by PC. The dialysates were concentrated, neutralized with barium carbonate, deionized with KU-2 cation-exchanger (H^+), evaporated again, and chromatographed. The partial hydrolysis of the PcSs for 2 and 3 h was conducted similarly.

Enzymatic Hydrolysis. A solution of 0.1 g of the pectin in 10 ml of water was treated with 0.01 g of pectinase. The mixture was incubated at 37°C for 48 h and the enzyme was then inactivated at 100°C for 5 min. The hydrolysis products were analyzed by PC.

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