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POLYSACCHARIDES OF *Ungernia*.
XV. A STUDY OF THE PECTIN SUBSTANCES OF THE LEAVES OF *Ungernia tadschicorum*

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Pectin substances have been isolated from the leaves of Ungernia tadschicorum *and characterized. A galacturonan has been obtained by partial hydrolysis. On the basis of the results of IR spectroscopy and periodiate — nitric acid oxidation, its structure has been established as an α-1→4-bound polymer-homolog.*

It has been established by an investigation of plants of the genus *Ungernia* (fam. Amaryllidaceae) that the predominating polysaccharides in the bulbs are water-soluble polysaccharides – natively acetylated mannans – and in the leaves they are pectin substances, which have been little studied in the chemical respect.

The leaves of *U. tadschicorum* contained 7% of pectin substances (PSs). The isolated PSs consisted of an amorphous cream-colored powder readily soluble in water, $[\alpha]_D^{20}$ + 132°, η_{rel} 13.8 (c 1.0; water), mol. mass 42,000. The galacturonic anhydride content was 64% and the neutral monosaccharides galactose, glucose, and rhamnose were found in a ratio of 3:3.2:1:1.5 [sic].

The presence of methoxy groups is an important index of pectin substances. To characterize the PSs we selected an arbitrary limit according to which all pectins containing up to 50% of methoxy groups are assigned to the low-esterification group, and about 50% of the high-esterification group [3]. The quantitative characteristics of the PSs studied were as follows (%): free carboxy groups, K_f – 6.6; methoxylated carboxy groups, K_e – 5.1; these figures show that the degree of methoxylation λ was 42%, and the $-O-CH_3$ content 2.8%. Thus, the results obtained permit the PSs to be assigned to the low-methoxyl group.

The IR spectra of the PSs contained the absorption bands characteristic for other pectins [4]: 3400, 2940, 1740, 1650, 1110, and 840 cm^{-1}.

By the partial acid hydrolysis of the PSs we obtained a galacturonan consisting solely of D-galacturonic acid residues. The galacturonan had a positive specific rotation $[\alpha]_D^{20}$ + 180° (c 1.0; 10% ammonia), which is characteristic for pectins. This gave grounds for the assumption that galacturonic acid residues in the pyranose form were linked by glycosidic bonds with the α-configuration. The presence of pyranose rings was confirmed by the frequencies of absorption bands in the IR spectrum of the galacturonan at 1730 cm^{-1} (stretching vibrations of the carbonyls of carboxy groups), 1030 and 1110 cm^{-1} (stretching vibrations of pyranose rings), and 830 cm^{-1} (α-glycosidic bonds). In order to establish the type of bond between the galacturonic acid residues, the partially methoxylated galacturonan was oxidized successively with periodic and nitric acids [7]. The formation, as the main oxidation product, of tartaric acid, which has four carbon atoms, showed that oxidation of α-diol groupings at the second and third carbon atoms of the galacturonic acid residues had taken place. This is possible when pyranose rings with the 1 → 4 type of bond between the monosaccharide residues are present.

On comparing the PSs isolated from other *Ungaria* species, it is possible to observe some differences in the quantitative contents of monosaccharides and in the degrees of esterification [6].

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EXPERIMENTAL

Paper chromatography was conducted on Filtrak-FN 11,12 paper in the solvent systems: 1) butan-1-ol–pyridine–water (6:4:3); and 2) butan-1-ol–acetic acid–water (4:1:5). For indicating the spots, we used: 1) acid aniline phthalate; and 2) aniline–glucose. The GLC of the samples was performed on a Chrom-1 instrument with a flame-ionization detector under the following conditions: stainless-steel column (0.3 × 200 cm), 5% of Silicone XE-60 on Chromaton NAW 0.200-0.250 mesh; 210°C; carrier gas helium, 60 ml/min. Aldononitrile acetates were obtained by a known procedure [7]. Samples of polysaccharides were hydrolyzed with 2 N H_2SO_4 at 100°C for 40-48 h.

The IR spectra of the samples were taken on a UR-20 instrument in KBr tablets. Specific rotations of the substances were determined in a Zeiss polarimeter, using a tube 1 dm long with a volume of 10 ml.

The quantitative determination of galacturonic anhydride was made by the colorimetric carbazole method [8]. A calibration curve plotted relative to galacturonic acid was used for the calculations. The quantitative characteristics of the pectin were determined by the titrimetric method [9]. The molecular masses of the PSs were determined viscosimetrically [10] in an Ostwald viscometer with a diameter of 0.73 mm.

Isolation of the Pectin Substances. The air-dry comminuted raw material (100 g) was treated with chloroform and was then dried and extracted with water (2 × 1 liter) at room temperature. The residual plant raw material was extracted with a mixture of 0.5% solutions of oxalic acid and ammonium oxalate (1:1) in a substance:solvent ratio of 1:20 at 70-80°C for 2-2.5 h. The extracts were dialyzed, evaporated, and treated with methanol, and the resulting precipitate was dried with acetone. Yield 7 g.

Partial Hydrolysis of the PSs. A solution of 1 g of the PSs in 150 ml of 0.5 N H_2SO_4 was heated at 100°C for 4 h. The precipitate that had deposited was separated off by centrifugation and was washed with 50% aqueous methanol and dried. Yield 0.45 g. When the product so isolated — a galacturonan — was rehydrolyzed (2 N H_2SO_4, 100°C, 48 h), D-galacturonic acid was detected with the aid of PC.

Preparation of a Galacturonan Partially Methyloxylated at the Carboxy Groups. A suspension of 1 g of the galacturonan in 20 ml of a 5% solution of dry HCl in absolute methanol was boiled on the water bath for 8 h. The methoxylated galacturonan was separated off by centrifugation, and was washed with alcohol and with acetone and was dried. The yield after two treatments amounted to 0.52 g $[\alpha]_D^{20}$ + 180° (c 0.4; water), $O-CH_3$–5.4%.

Oxidation of the Partially Methoxylated Galacturonan. A solution of 0.5 g of the substance in 50 ml of water was treated with 3 g of crystalline periodic acid in 20 ml of water. Oxidation was conducted in the dark at 20-22°C for 80 h. The excess of periodic acid was eliminated by means of an anion-exchange resin, the solution was evaporated to dryness, and an amorphous cream-colored powder was obtained in a yield of 0.21 g.

Hydrolysis of the Semialdehyde. The semialdehyde (50 mg) was hydrolyzed with 5 ml of 1 N H_2SO_4 in a sealed tube for 20 h. PC (system 1, revealing agent 1) showed a very small amount of galacturonic acid.

The oxidation of the semialdehyde with nitric acid was carried out as described in [5]. In the oxidation products, after acid hydrolysis, oxalic and tartaric acids were detected by PC (system 2, revealing agent 2). The oxalic acid was obtained in the crystalline form, mp 166-167°C, giving no depression with an authentic sample.

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