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POLYSACCHARIDES OF *Ungernia*.

X. PECTINS FROM THE LEAVES OF *Ungernia sewerzowii*

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The characteristics of the pectin from the leaves of *Ungernia sewerzowii* are given. The pectin isolated consists of residues of: galacturonic acid — 51%; rhamnose — 23.6%; arabinose — 4.9%, xylose — 1.3%; glucose — 4.1%; and galactose — 15.3%. $[\alpha]_D^{25}$ + 206° (c 0.5; water, OCH_3 — 5.4%. Partial hydrolysis of the pectin gave a galacturonan containing only galacturonic acid residues. On the basis of the results of periodate–silver nitrate oxidation and methylation it was shown that the galacturonic acid residues in the galactone are linked by α-1 → 4-glycosidic bonds.

It has been found previously [1] that the predominating polysaccharides of the leaves of *Ungernia sewerzowii* (Rgl.) B. Fedsch. are pectin substances, and those of the bulbs are reserve polysaccharides: a natively acetylated mannan and starch. We have determined the amount of carbohydrates in the leaves of *Ungernia sewerzowii* gathered on April 16, 1976 in Galvasae, Tashkent province. The polysaccharides were extracted successively from one sample of air-dry material: first the water-soluble polysaccharides [2], and then the pectin substances [3] and the hemicelluloses (Table 1).

The pectin substances have been studied in more detail. The pectins isolated consisted of a cream-colored powder readily soluble in water, $[\alpha]_D^{25}$ + 206° (c 0.5; water). The amount of galacturonic anhydride determined by a standard method [4] was 51%, and the amount of OCH_3 5.4%. The percentages of the neutral monosaccharides were as follows: rhamnose, 23.6; arabinose, 4.9; xylose, 1.3; glucose, 4.1; and galactose, 15.3. The galacturonic acid, which was isolated in the pure state, was identified by oxidation to mucic acid with mp 215-216°C [5].

The quantitative characteristics of the pectin obtained by a titrimetric method [6] were (%): free carboxy groups, K_f — 11.5; methoxylated carboxy groups K_e — 7.1; degree of methoxylation, λ — 38.

The IR spectrum of the pectin contained the absorption bands that are characteristic for other pectins [7]: 3420, 2950, 1750, 1640, 1120, 1020, and 840 cm^{-1}.

The molecular weight, determined viscosimetrically [8] was 54,000 a.u., and that calculated from the sedimentation constant was 49,000 a.u.

The periodate oxidation of the pectins was carried out in a neutral medium. The consumption of sodium periodate amounted to 0.53 mole per mole of anhydrohexose per unit. On Smith

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TABLE 1. Amounts and Monosaccharide Compositions of the Carbohydrates from the Leaves of *Ungernia sewerzowii*

Type of polysaccharide	Yield of polysaccharide	Ratio of neutral monosaccharides							GalUA, %
		Rha	Ara	Xyl	Man	Glc	Gal	Fru	
Water-soluble polysaccharide	5,6	6,4	10,6	1	2,7	37,5	29	—	Tr.
Glucofructan	7,2	—	Tr.	Tr.	—	+	Tr.	+	—
Pectin substances	7	17,9	3,7	1	—	3,1	11,6	—	51
Hemicellulose I	5,2	6,6	4,6	10,5	1	8	12,8	—	+
Hemicellulose II	1,7	1	2,3	8 3	1,4	7,9	4	—	+

degradation [9], rhamnose, arabinose, galactose, glycerol, and erythritol were detected by PC and GLC in a ratio of 1 : 1.4 : 1.5 : 4.6 : 24.2, and galacturonic acid was also detected. Consequently, the low consumption of sodium periodate in the presence of the unoxidized monosaccharides indicates a branched structure of the pectin.

Partial hydrolysis of the pectin yielded a polysaccharide (galacturonan) containing only galacturonic acid residues. Its IR spectrum had absorption bands at 3400, 2930, 1750, 1630, 1410, 1340, 1230, 1110, 1020, 950, 890, and 830 cm^{-1}. The pyranose nature of the rings was confirmed by the presence of the absorption bands at 1020 and 1110 cm^{-1} (ring vibrations of pyranoses and C—O vibrations). The high positive specific rotation of $[\alpha]_D^{25}$ + 272° (c 0.25; water) permits the assumption that the glycosidic bond between the galacturonic acid residues in the pyranose form has the α configuration.

To establish the positions of the bonds, the previously methoxylated galacturonan was oxidized successively with periodic and nitric acids [10]. Oxalic and tartaric acids were isolated in the oxidation products by PC. The formation of the latter showed that α-diol groupings at the second and third carbon atoms had undergone oxidation. This is possible only on the case of 1 → 4 bonds between the galacturonic acid residues.

As is well known, the methylation of a galacturonan is difficult, and therefore it was first reduced to a galactan and was then methylated, or methylation by Hakomori's method [11] and reduction with $LiAlH_4$ [12] were alternated. This gave a completely methylated galactan with c $[\alpha]_D^{24}$ + 9° (c 1%; $CHCl_3$). On complete acid hydrolysis of a permethylate, 2,3,6-tri-O-methylgalactose and 2,3,4,6-tetra-O-methylgalactose were detected by TLC with markers. The 2,3,6-tri-O-methylgalactose obtained as the main product shows the presence of 1 → 4 bonds in the galactan, and therefore, also in the galacturonan.

EXPERIMENTAL

Paper chromatography was performed on Filtrak-FN-11,3 paper in the following solvent systems: 1) butan-1-ol—pyridine—water (6 : 4 : 3); 2) butan-1-ol — acetate acid — water (4 : 1 : 5). Thin-layer chromatography (TLC) was performed on Silufol plates of type UV-254 in the solvent systems 3) benzene—acetone (1 : 1) and 4) chloroform—methanol (10 : 1). The following reagents were used to indicate the spots: 1) aniline hydrogen phthalate; 2) periodate—$KMnO_4$—benzidine; and 3) aniline—glucose. The GLC of the samples was performed on a Tsvet-101 instrument with a flame-ionization detector under the following conditions: steel column (200 × 0.3 cm), 5% of Silicone XE-60 on Chromaton NAW (0.200-0.250 mm), 210°C, carrier gas helium at the rate of 60 ml/min. The acetates of the aldononitriles were obtained by the method of Bouveng et al. [13]. Samples of the polysaccharides were hydrolyzed with 2 N H_2SO_4 at 100°C for 40-48 h. IR spectra were recorded on a UR-20 instrument in paraffin oil and in tablets of KBr.

Isolation of the Polysaccharides. A 100-g sample of the air-dry comminuted raw material was treated with chloroform and extracted with water (1 liter × 2) at room temperature. The extracts were evaporated and precipitated with methanol. This gave 5.6 g of water-soluble polysaccharide. The methanolic solution gave 7.2 g of glucofructan. The residue of the plant raw material was extracted with a mixture of 0.5% solutions of oxalic acid and ammonium oxalate (2 × 1 liter) at 70°C. From the extract methanol precipitated 7.0 g of pectin. The residual material was extracted successively with 5 and 10% solutions of NaOH at room temperature. After dialysis, extracts were evaporated and precipitated with methanol. This gave hemicelluloses I and II in amounts of 5.2 and 1.7 g, respectively.

Identification of the Galacturonic Acid. The pectin (0.5 g) was hydrolyzed with 12 ml of 2 N H_2SO_4 on the boiling water bath for 48 h, and the reaction mixture was neutralized with barium carbonate and filtered. The galacturonic acid in the form of a barium salt was precipitated with methanol. The precipitate was separated off, carefully washed with methanol, and dissolved in water, and the solution was treated with KU-2 cation-exchange resin. Only galacturonic acid was found by PC (in system 1). Bromine was added to an aqueous solution of the galacturonic acid until it was saturated, and the mixture was left for 48 h. Evaporation of the solution yielded 100 mg of mucic acid, mp 215-216°C. It gave no depression of the melting point with an authentic sample.

Periodate Oxidation and Smith Degradation of the Pectin. A solution of 100 mg of the pectin in 33 ml of water was treated with 7 ml of 0.2 M sodium periodate solution and the mixture was left in the dark at room temperature. Aliquots with a volume of 1 ml were taken and the excess of sodium periodate was titrated with 0.01 N sodium thiosulfate solution. After 34 days, the consumption of sodium periodate amounted to 0.53 mole and did not change further. After the decomposition of the periodate and dialysis the oxidation product was reduced with sodium tetrahydroborate and was hydrolyzed with 2.5 ml of 1 N H_2SO_4 at 100°C for 8 h, and PC then showed the presence of galacturonic acid, galactose, arabinose, and rhamnose (system 1, visualizing agent 1) and glycerol and erythritol (system 1, visualizing agent 2). Part of the hydrolysate was analyzed by GLC. Rhamnose, arabinose, galactose, glycerol, and erythritol were detected in a ratio of 1 : 1.4 : 1.5 : 4.6 : 24.2.

Partial Hydrolysis of the Pectin. A mixture of 3.0 g of the pectin and 150 ml of 2 N H_2SO_4 was heated on the boiling water bath for 8.5 h. The resulting precipitate was centrifuged off and it was washed repeatedly with 80% methanol and was then dissolved in water, and dialyzed. Then the solution was evaporated and precipitated with methanol. The yield of galacturonan was 1.1 g, $[\alpha]_D^{25}$ + 272° (c 0.25; water). On total acid hydrolysis, PC (system 1, visualizing agent 1) showed the presence only of galacturonic acid.

Periodate—Nitric Acid Oxidation of the Galacturonan. A suspension of 1.0 g of the galacturonan in 15 ml of 5% solution of dry HCl in absolute methanol was boiled on the water bath for 8 h. This gave 0.45 g of partially methoxylated galacturonan with $[\alpha]_D^{20}$ + 225° (c 0.5; water), which was then oxidized with periodic acid (3 g in 18 ml of water) and with concentrated nitric acid (5 ml) [10]. In the oxidation products after acid hydrolysis, PC (system 2, visualizing agent 3) showed the presence of oxalic and mucic acids. The latter was obtained in the crystalline form with mp 165-167°C, $[\alpha]_D^{20}$ − 12° (c 0.5; water). It gave no depression of the melting point with an authentic sample.

Methylation of the Galacturonan. The galacturonan (0.5 g) was methylated by Hakomori's method [11]. The partially methylated galacturonan in dry tetrahydrofuran (20 ml) was heated with $LiAlH_4$ (0.15 g) for 18 h. Then the excess of reducing agent was decomposed with water. After dialysis, the filtered solution was evaporated and precipitated with three volumes of methanol. The methylation and reduction procedures were repeated. Finally, methylation was carried out by Hakomori's method. A methylated galacturan was obtained; 0.02 g, $[\alpha]_D^{24}$ + 9° (c 1; $CHCl_3$). The IR spectrum lacked the hydroxyl absorption band. The permethylate was hydrolyzed by the method of Bouveng et al. [14], and 2,3,4,6-tetra-O-methylgalactose and 2,3,6-tri-O-methylgalactose with R_f 0.25 and 0.19, respectively, were detected by GLC (systems 3 and 4, visualizing agent 1).

SUMMARY

1. The amounts of carbohydrates in the leaves of *Ungernia sewerzowii* (Rgl.) B. Fedtsch. have been determined. The qualitative and quantitative monosaccharide compositions of the polysaccharides have been established.

2. A galacturonan has been obtained by the partial hydrolysis of the pectin. On the basis of the results of periodate—acetic acid oxidation and methylation it has been shown that in the galacturonan the galacturonic acid residues are linked by α-1 → 4 glycosidic bonds.

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NEUTRAL LIPIDS OF THE SEEDS OF *Helleborus abchasicus*

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The neutral lipids of seeds of *Helleborus abchasicus* of the second vegetation phase have been studied. Fatty acid methyl esters have been found in the seed oil of *Helleborus abchasicus*. In a study of the triacylglycerols it has been established that the predominating acid in the acylglycerols is linolenic, and the main species of triacylglycerols are two-acid species with oleic and linolenic acids in position 2.

We have previously studied the chemical composition of the lipids of the roots of rhizomes of *Helleborus abchasicua* A Br. (Abkhazian hellebore; family Ranunculaceae), which is a plant endemic for the Georgian SSR and which in experiments on animals exhibits antitumoral activity [1, 2]. This plant vegetates twice a year. It has been shown that the compositions of the lipids of the epigeal organs of the two phases of vegetation are different [3].

In the present paper we give the results of a study of the chemical composition of the seeds of *H. abchasicus* from the second vegetation phase.

The seeds of the *H. abchasicus* contained 30% of lipids which consisted of an oily yellow liquid with a characteristic unpleasant odor. Some physicochemical indices of the lipids were determined: d_{20}^{20} 0.9019; acid No. 149 mg KOH; n_D^{20} 1.4729; iodine No. 152.0%.

To isolated the total fatty acids, the lipids were hydrolyzed, and the acids were converted into their methyl esters, which were analyzed by GLC on polar and medium-polar phases (Table 1).

It can be seen from Table 1 that the lipids of the *H. abchasicus* seeds have a fairly complicated fatty-acid composition. The polar phase gives good separation of the 18 : 2 and 18 : 3 components. On the moderately polar phase OV-101, the 18 : 2 and 18 : 3 components are superposed on one another but the 8 : 0, 9 : 0, 10 : 0, 11:0, 12 : 0, 13 : 0, 14 : 0, and 15 : 0 acids appear, in addition, and the total unsaturated acids decrease. We cannot explain the differences in the amounts of the 20 : 1 acid obtained with Reoplex and ethylene succinate nor the differences in the amounts of the 15 : 0, 18 : 1, and 18 : 2 acids obtained on the OV-101, as compared with the other stationary phases.

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