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The pectin substances have been isolated from *Eremurus regelii* Vved., their qualitative and quantitative monosaccharide compositions have been determined, and their physicochemical properties have been studied. The partial hydrolysis of the pectin gave a galacturonan. On the basis of the results of periodate-nitric acid oxidation, enzymatic hydrolysis, and methylation it has been shown that in the galacturonan the galacturonic acid residues are linked by α -(1 \rightarrow 4)-glycosidic bonds.

The predominating carbohydrate components of the leaves of *Eremurus regelii* Vved. are pectin substances [1]. In the present paper we give their characteristics and information on the structure of the galacturonan.

The pectin substances were isolated by a known method [1]. The pectin formed a fibrous powder with a light cream color readily soluble in water, $[\alpha]_D^{20} + 180^\circ$ (c 0.2; 0.1 N NaOH). The amount of uronic anhydride was 65.7% and that of OCH_3 3.8%. The percentage amounts of the neutral monosaccharides were as follows: rhamnose 23; arabinose, 3.3; xylose, 1.17; galactose 6.33. The quantitative characteristics of the pectin found by the titrimetric method [2] were (%): free carboxy groups, K_c 13.7; methoxylated carboxy groups, K_e , 2.3; degree of methoxylation, λ , 14.35. It can be seen from the titrimetric results that the pectin had a low degree of methoxylation. The molecular weight calculated from its sedimentation content was 79,500 c.u.

Partial hydrolysis of the pectin gave a galacturonan (yield 40%). Only galacturonic acid was found in the products of the acid and enzymatic hydrolysis of the galacturonan. The IR spectrum of the galacturonan had absorption bands at 830, 890, 950, 1020, 1110, 1230, 1340, 1410, 1630, 1750, 2930, and 3400 cm^{-1} .

The presence of a pyranose ring could be provisionally assumed from the absorption in the IR spectrum at 1020 and 1110 cm^{-1} (ring vibrations of pyranoses and C=O vibrations).

The high positive specific rotation of the galacturonan $[\alpha]_D^{20} + 220^\circ$ (c 0.2; 0.1 N NaOH) also permitted the assumption that glycosidic bonds between the galacturonic acid residues in the pyranose form had the α configuration. To prove the position of the bonds between the galacturonic acid residues, the partially esterified galacturonan was subjected to periodate-nitric acid oxidation [3]. Oxalic and tartaric acids were identified in the oxidation products by PC. The formation of the latter shows that α -diol groupings at the second and third carbon atoms had been subjected to oxidation. This is possible only in the case of a 1 \rightarrow 4 bond between the galacturonic acid residues.

The methylation of the galacturonan took place with difficulty, and it was therefore esterified with diazomethane, reduced with sodium tetrahydroborate to a galactan [4], and methylated by Hakomori's method [5] and then by Purdie's [6] method. A galactan permethylate was obtained with $[\alpha]_D^{20} + 16^\circ$ (c 1.5; CHCl_3). In the products of the complete acid hydrolysis of the permethylate by TLC with markers showed the presence of 2,3,6-tri-O-methylgalactose and 2,3,4,6-tetra-O-methylgalactose. The formation of 2,3,6-tri-O-methylgalactose as the main product showed the presence of 1 \rightarrow 4 bonds in the galacturonan.

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EXPERIMENTAL

Descending paper chromatography was performed on Filtrak FN 11 paper in the following solvent systems: 1) butan-1-ol-pyridine-water (6:4:3), and 2) butan-1-ol-acetic acid-water (4:1:5). Thin-layer chromatography (TLC) was carried out on Silufol UV-254 plates in the following systems: 3) benzene-acetone (1:1), and 4) chloroform-methanol (10:1).

To indicate the spots we used the following reagents: 1) aniline hydrogen phthalate, and 2) 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% Silicone XE-60 on Chromaton NAW (0.200-0.250 mesh), column temperature 210°C, carrier gas helium at the rate of 60 ml/min. Aldononitrile acetates were obtained as described by Ovodov [7].

Samples of the pectin substances were hydrolyzed with 2 N H₂SO₄ at 100°C for 48 h.

IR spectra were taken on a UR-20 instrument in paraffin oil and in tablets with KBr. Ultracentrifugation was performed on a MOM-3170 instrument at 50,000 rpm; temperature 20°C, exposure interval 5 min, angle of rotation 45 degrees. A 1% aqueous solution of the substance was used. The molecular weight found was 79,500.

Isolation of the Pectin Substances. The ground air-dry raw material collected in the village of Kaplanbek in the fruit-bearing stage (200 g) was treated with chloroform and was then extracted with water to eliminate the water-soluble polysaccharide. The pectin substances (PSS) were extracted with a mixture of 0.5% solutions of oxalic acid and ammonium oxalate in a ratio of 1:10 at 70°C. The extract was dialyzed, and the addition of methanol then precipitated 34 g of pectin. The products of the complete acid hydrolysis of the PSS were found by PC (system 1, revealing agent 1) to contain rhamnose, arabinose, xylose, galactose, and galacturonic acid. Another part of the hydrolysate was analyzed by TLC, and rhamnose, arabinose, xylose, and galactose were detected in a ratio of 19.7:3.16:1:5.4 (in moles). $[\alpha]_D^{20} + 180^\circ$ (c 0.2; 0.1 N NaOH).

Preparation of the Galacturonan. A mixture of 4 g of the pectin and 200 ml of 2 N H₂SO₄ was heated in the boiling water bath for 4 h. The precipitate was centrifuged off, washed with 1% H₂SO₄ and 80% ethanol, dissolved in water, and then was dialyzed. The it was evaporated and precipitated with ethanol. The yield of galacturonan was 1.6 g $[\alpha]_D^{20} + 220^\circ$ (c 0.25 0.1 N NaOH). On complete acid hydrolysis of the galacturonan, galacturonic acid was revealed by PC (system 1, revealing agent 1).

Enzymatic Hydrolysis of Galacturonan. A solution of 0.1 g of the galacturonan in 10 ml of water was treated with 0.01 g of pectinase (Fluka) at pH 4. The mixture was incubated at 37°C for 48 h and was then boiled for 5 min to stop the enzymatic hydrolysis. The products of enzymatic hydrolysis were analyzed by PC (system 1, revealing agent 1), and galacturonic acid was detected.

Periodate-Nitric Acid Oxidation of the Galacturonan. A suspension of 1 g of the galacturonan in 15 ml of a 5% solution of dry HCl in absolute methanol was boiled in the water bath for 8 h. This gave 0.74 g of a partially methylated galacturonan $[\alpha]_D^{20} + 187^\circ$ (c 0.1; H₂O), which was then oxidized with periodic acid (4.6 g in 26 ml of water) and concentrated nitric acid (7 ml) [3]. Oxalic and tartaric acid were detected by PC (system 2, revealing agent 2) in the products of the oxidation of the galacturonan after acid hydrolysis. The tartaric acid was obtained in the crystalline form with mp 167-169°C (ethanol-ether).

Preparation of the Galactan. A 2-g sample of the galacturonan was ground to a powder and was moistened with 70% methanol, after which a solution of diazomethane in ether was added. The mixture was kept at +4°C for 24 h, and then the galacturonan was filtered off and washed with ether.

The product obtained was dissolved in 75 ml of water and was reduced with 1 g of sodium tetrahydroborate as described in [4]. As the result of esterification and reduction (ten times) of the galacturonan, a galactan was obtained with a yield of 0.302 g (15.1%).

Methylation of the Galactan. The galactan (0.302 g) was methylated by Hakomori's method [5]. Methylation was completed by Purdie's method [6]. This gave a methylated galactan with $[\alpha]_D^{20} + 16^\circ$ (c 1.5; CHCl₃). The IR spectra lacked the absorption band of hydroxyls. The permethylate was hydrolyzed by the method of Boveng et al. [8]. TLC with markers (systems 3 and 4, revealing agent 1) showed the presence of 2,3,4,6-tetra-O-methylgalactose and of 2,3,6-tri-O-methylgalactose (intense spot).

SUMMARY

The qualitative and quantitative monosaccharide composition and the physicochemical properties of the pectin substances from the leaves of *Eremurus regelii* Vved. have been studied. A galacturonan has been obtained by the partial acid hydrolysis of the pectin. On the basis of the results of periodate-nitric acid oxidation, enzymatic hydrolysis, and methylation, it has been shown in the galacturonan the galacturonic acid residues are linked by α -(1 \rightarrow 4)-glycosidic bonds.

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CARBOHYDRATES OF *Allium*.

IX. CARBOHYDRATE COMPONENTS OF *Allium cepa*

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The carbohydrate components of the bulbs of *Allium cepa* L. (white variety) have been characterized. It has been established that the main carbohydrate component is the ethanol-soluble fraction. Information has been obtained on the elementary compositions and origin of a number of fragments in the mass spectrum of the peracetate of 1^F-kestose.

We have previously described the ethanol- and water-soluble components of the bulbs of *Allium cepa* L. (red variety) [1]. In the present paper we consider the carbohydrate components of the white variety of this species. In contrast to the red variety, here we observe that the ethanol-soluble fraction is the main carbohydrate component.

By successive extraction of the bulbs we obtained the ethanol-soluble fraction (80% ethanol) (ES) (75.5%, on the absolutely dry raw material), the water-soluble polysaccharides (WSPSs) (6%), the pectin substances (PSSs) (4.9%), and the hemicelluloses (HMCs) (0.17%).

The ES fraction, after purification with carbon, was analyzed by paper and thin-layer chromatography, with the detection of fructose, glucose, sucrose, and oligosaccharides with R_f 0.08 and 0.2 remaining in the ethanol.

When the ES material was fractionated on a column of Sephadex G-15, ten fractions, ES-I-ES-X, were obtained; their characteristics are given at the top of following page.

On comparing their chromatographic mobilities on PC and TLC, their molecular weights, and their specific rotations, ES-X and ES-IX were identified as fructose, glucose, and sucrose, respectively.

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