PECTIN SUBSTANCES OF Chara aculeolata

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We have previously shown the presence in the carbohydrate complex of Chara aculeolata Kütz (a Chara alga) of a glycanogalacturonan in addition to starch and cellulose [1]. We have since studied the structure of the acid polysaccharide of this raw material.

The polysaccharide was isolated from the alga by extraction with a solution of ammonium oxalate and was purified by reprecipitation with ethanol followed by amylolysis to remove possible reserve glucan impurities. On the basis of the results of hydrolysis and chromatography it was found that it was constructed mainly of residues of a uronic acid. By PC in comparison with markers, and also by reduction to galactose it was shown that this acid was D-galacturonic acid. In a hydrolyzate of the polysaccharide we found (% by weight of the samples): galacturonic acid (by decarboxylation 90.5, by the carbazole method 78.8), glucose (2.2), arabinose (1.2), and xylose (0.2). The pectin substances of the Chara alga [α]D +290.8°, molecular weight 24,000 carbon units) contained 0.01% of nitrogen, 4.40% of ash, and, as functional groups (% on the sample) free COOH 21.09, OMe 1.02, and OAc.

The high content of D-galacturonic acid residues, the presence in the polysaccharide of methoxy groups and inorganic compounds, and the considerable positive specific optical rotation permitted it to be assigned to the category of pectin substances. The pectin of Chara aculeolata is distinguished by an insignificant degree of esterification of the carboxy groups and contains no ester-bound sulfate, which is characteristic for many algal polysaccharides [2]. The pectin was precipitated completely by Cetavlon and by aluminum sulfate. According to the results of chromatography on Sephadex C-75, 100, and 150, it is uniform in molecular weight. On electrophoresis in phosphate buffer the whole of the polysaccharide migrated in the direction of the anode. Fractionation on DEAE-cellulose showed the presence of one fraction of acid polysaccharide eluted by alkali. All this shows the homogeneity of the pectin isolated.

The IR spectrum of the substances was similar to the spectra of the pectins of higher plants.

The pectin was 78% decomposed by the action of pectinase. The products of enzymatic hydrolysis were found to contain — in addition to D-galacturonic acid — all the neutral monosaccharides present in the substance and oligomers having an acid nature. The latter were separated by preparative PC and subjected to additional acid hydrolysis. The resulting hydrolyzates were found to contain only the D-galacturonic acid residues forming the main homogeneous chain of the polysaccharides.

We have studied the structure of the main polyuronide fragment of the pectin molecule — a galacturonan — obtained by the partial acid hydrolysis of the initial substances. It contained 98% of D-galacturonic acid and 1.01% of OCH3; [α]D28 +260.8°; molecular weight 6000 carbon units. Like the initial pectin, its fragment had a high positive specific rotation. It consisted only of D-galacturonic acid residues esterified to a very small extent and was characterized by a molecular weight considerably smaller than that of the initial pectin.

To determine the structure of the galacturonan we used methylation and enzymatic hydrolysis. Since the methylation of the galacturonan took place with difficulty, the polysaccharide was first reduced to the galactan. Methylation was performed by Hakomori's method. According to chromatography on Al2O3, methylation took place exhaustively. By PC,
2,3,6-tri-O-methyl-D-galactose and very small amounts of 2,3,4,6-tetra-O-methyl-D-galactose were identified in the hydrolyzate of the modified product. In parallel, the products of methanolysis were found by GLC and TLC in layers of impregnated silica gel to contain methyl 2,3,6-tri- and 2,3,4,6-tetra-O-methyl-D-galactosides, the latter in trace amounts. No compounds with a low degree of methylation were found. This shows that the galactan and, consequently, the initial galacturonan, contains an unbranched chain of residues of D-galacturonic acid. The considerable positive specific rotation of the polysaccharides shows the α configuration of the glycosidic centers. In the galacturonan, the D-Galacturonic acid residues are present in the pyranose form, as is confirmed by its IR spectrum which has absorption bands at 1050 and 1080 cm⁻¹ (the ring vibrations of pyranoses and C-O) and at 745 and 925 cm⁻¹ (symmetrical and asymmetrical vibrations of a pyranose ring). An additional proof of an α-1→4 bond between the D-galacturonic acid residues in the galacturonan was its high susceptibility to attack by pectinase. In contrast to the initial pectin it was cleaved almost completely.

Thus, like the pectins of higher plants, the main fragment of the pectin of *Chara aculeolata* — a galacturonan — is linear and consists of D-galacturonic acid residues in the pyranose form linked through the 1→4 carbon atoms.

**EXPERIMENTAL**

**Isolation of the Pectin.** Algae of the species *Chara aculeolata* Kütz were collected in Egorlyk Bay, Black Sea. The carefully purified and comminuted raw material was extracted with a mixture of methanol and benzene in a Soxhlet apparatus. The residue was washed with water at 90°C to eliminate water-soluble polysaccharides and the remaining 100 g was treated with a 0.25% solution of ammonium oxalate in a 0.25% solution of oxalic acid. The pectin was precipitated from the extract with ethanol. The polysaccharide was purified by reprecipitation from solution in ammonium oxalate. It was freed from inorganic impurities by dialysis. Yield 3 g.

**Hydrolysis.** The pectin was hydrolyzed with 2 N H₂SO₄ in sealed tubes at 100°C for 12 h. The monosaccharides in the hydrolysate were identified by paper chromatography in comparison with markers. As the mobile solvent we used pyridine–butanol–water–benzene (3:5:3:1) and ethyl acetate–acetic acid–formic acid–water (18:3:1:4). The spots were revealed with aniline phthalate. The monomeric composition of the pectin is given above.

**Characteristics of the Pectin.** The molecular weight was determined viscosimetrically [3]. The material was fractionated on DEAE-cellulose treated by the method of Neykom et al. [4]. The fractions were monitored by the carboxyl and anthrone methods.

**Isolation of the Galacturonan.** The galacturonan was isolated by R. G. Ovodova's method [5]. Yield 39.4%.

**Reduction of the Galacturonan.** Since methylation of the galacturonan took place with difficulty, it was previously methoxylated with diazomethane and reduced with sodium tetrahydroborate to the corresponding galactan [6].

The galactan was methylated by Hakomori's method [7]. A fully methylated product was obtained.

**Hydrolysis of the Methylated Galactan.** The methylated galactan was formolized with 90% formic acid at 100°C for 1 h and was then hydrolyzed with 0.25 M H₂SO₄ at the same temperature for 14 h. The hydrolysis products were identified by the PC method. 2,3,6-Tri-O-methyl-D-galactose and traces of 2,3,4,6-tetra-O-methyl-D-galactose were found. Part of the methylated galactan was subjected to methanolysis with a 4% solution of hydrochloric acid in methanol. The methyl galactosides were identified by TLC and GLC. Methyl 2,3,6-tri-O-methyl-D-galactoside and a very small amount of methyl 2,3,4,6-tetra-O-methyl-D-galactoside were found.

**Enzymatic Hydrolysis.** The pectin was treated with a preparation of pectinase isolated from culture liquid of the fungus *Aspergillus awamori*, in water at 37°C. In parallel, as a control, apple pectin was hydrolyzed by the pectinase. Its degree of cleavage amounted to 85%.