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 $[\alpha]_D^{20}$ +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 OCH_3; $[\alpha]_D^{20}$ +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 Al_2O_3, methylation took place exhaustively. By PC,

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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 H_2SO_4 in sealed tubes at 100°C for 12 h. The monosaccharides in the hydrolyzate 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 carbazole 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 H_2SO_4 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%.

SUMMARY

The carbohydrate complex of the Chara alga *Chara aculeolata* Kütz includes an acid polysaccharide similar to the pectin substances of higher plants. It is based on a fragment constructed of α-1→4 bound residues of D-galacturonic acid in the pyranose form. The pectin is characterized by a high homogeneity, a considerable content of D-galacturonic acid, and a low degree of acidification of the carboxy groups.

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EPOXY ACIDS OF THE SEED OIL OF *Artemisia absinthium*

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The seed oil of *Artemisia absinthium* (common wormwood) has previously been found to contain about 15% of epoxy acids, on the total acids present in the form of acyl radicals in the triglycerides [1, 2]. The unusually high content of epoxy acids attracted our attention to this source of vernolic and coronaric acids. However, in the seed oil of plants of this species growing in Central Asia we detected no signals of the protons of an epoxide ring by nuclear magnetic resonance (NMR) while at the same time the IR spectrum of the oil showed a region of absorption of the vibrations of the bonds of an epoxide ring. The reason for this may be a content of epoxy acids considerably smaller than 15%.

To determine the concentration more accurately and for use as standards in the investigation of other plants we studied the structure of the epoxy acids of the seed oils of the Central Asian wormwood. The oil was subjected to transesterification with methanol in the presence of sodium methoxide and the mixture of methyl esters (MEs) of the fatty acids was isolated. By thin-layer chromatography (TLC) on Silufol plates (system 1) followed by treatment with picric acid, the mixture obtained was found to contain two sharp zones of the MEs of epoxy acids. Then the mixture of MEs (50 g) was transferred to a column 3 cm in diameter filled with silica gel (100 mesh) to a height of 10-15 cm. The MEs of the unsubstituted fatty acids were eluted with light petroleum ether (the process was monitored on Silufol plates in system 1).

The methyl esters of the oxy acids (acids in which an atom or atoms of hydrogen are the aliphatic chain are replaced by oxygen or a hydroxy group) were eluated from the column with diethyl ether. The resulting concentrate of oxy acids, containing MEs of unsubstituted fatty acids as impurities, was separated in an ascending chromatographic column (system 1). This gave two fractions of MEs of epoxy acids (Fig. 1a), each of which was subjected to TLC on silica gel (100 mesh) impregnated with 20% of silver nitrate in system 2. When the chromatograms were treated with iodine, sulfuric acid, and the picric acid reagent, a slowly moving zone was found to contain a mixture of MEs of unsaturated epoxy acids (II) and traces

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