THE STRUCTURE OF THE POLYURONIDE OF THE LEAVES OF THE SUGAR BEET

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The structure of the polygalacturonides of the leaves of various plants has been studied to a comparatively small extent [1]. The characteristics of the polyuronide of the leaves of the sugar beet are of interest in various ways [2].

We have studied the structure of the polygalacturonide isolated from the leaves of monogerm sugar beet of the variety Yaltushkovskaya. The soluble and the insoluble pectin (protopectin) were isolated from the raw material by E. V. Sapozhnikova's method [3]. These products were chromatographed on DEAE-cellulose (Fig. 1). The water-soluble pectin consisted of one neutral fraction and two acidic fractions, and the water-insoluble pectin consisted of three acidic fractions.

The amounts of the individual fractions and their monosaccharide compositions can be judged from the figures given in Table 1. The neutral polysaccharide of the water-soluble pectin is constructed solely from arabinose and galactose residues. The other polysaccharides contain galacturonic acid residues in various ratios and some other monosaccharides.

Thus, the pectins of sugar beet leaves, like a number of others [3, 4], are a mixture of acidic polysaccharides.

Then, using periodate oxidation, Smith degradation, and methylation, we studied the structure of the main fragment of which the protopectin molecule is constructed, i.e., a polygalacturonide. Since the methylation of polygalacturonides takes place with difficulty, the polysaccharide was first methoxylated and reduced to the corresponding galactan. Its yield amounted to 55%.

By the exhaustive periodate oxidation of the galactan followed by tetrahydroborate reduction of the polyaldehyde we obtained a polyalcohol. After hydrolysis, threitol and glycerol were identified in the solution by chromatography. The formation of threitol was possible only if there are 1→4 bonds between the galactopyranose residues.

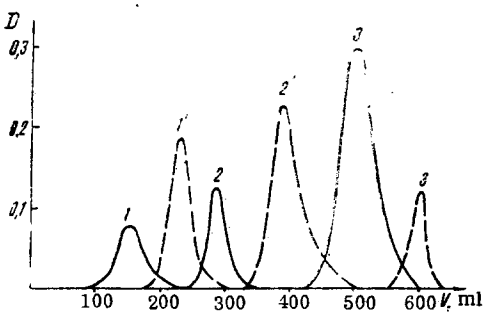

Fig. 1. Chromatograms of the soluble pectin (1, 2, 3) and of the protopectin (1', 2', 3') on DEAE-cellulose.

The galactan was methylated by Hakomori's method. The chromatography of the methylated polysaccharide on Al_2O_3 gave only one spot. This shows its homogeneity. The IR spectra taken did not contain absorption bands in the hydroxy group region. This shows that the methylation process had gone to completion.

A hydrolyzate of the compound obtained was shown by paper chromatography to contain 2,3,4,6-tetra- and 2,3,6-tri-O-methyl derivatives of galactose.

The gas–liquid chromatography of the products performed in parallel showed that they contained methyl 2,3,4,6-tetra- and 2,3,6-tri-O-methyl-D-galactosides. The amount of 2,3,6-tri-O-methyl-D-galactose in the hydrolyzate

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TABLE 1. Monosaccharide Composition of Fractions of Pectins Obtained by Chromatography on DEAE-cellulose

Fraction No.	Elution conditions	Yield of the fraction, %	Monosaccharide composition, %				
			galacturonic acid	arabinose	galactose	xylose	rhamnose
	Water-soluble pectin						
1	Water	13,9	—	61,4	39,1	—	—
2	0,5 M. NaH_2PO_4	19,3	48,3	31,7	10,4	4,5	3,3
3	0,1 M. NaOH	64,7	89,9	6,3	—	—	—
	Protopectin						
1	0,1 M. NaH_2PO_4	33,3	29,1	40,6	17,9	—	2,4
2	0,5 M. NaH_2PO_4	39,6	55,4	30,1	9,6	3,1	Traces
3	0,1 M. NaOH	23,4	77,6	21,6	—	—	—

was considerably greater than the amount of the other methylated derivatives. No compounds with a low degree of methylation were found. This shows that the galactan and, consequently, the initial polygalacturonan contained an unbranched chain of galactose (galacturonic acid) residues.

The considerable positive specific optical rotation of the polysaccharide showed the α-configuration of the glycosidic bonds.

In the molecule, the galacturonic acid residues are present in the pyranose form. This is shown by the IR spectra which have absorption bands at 1050 and 1080 cm^{-1} (ring vibrations of pyranoses and C–O vibrations) [5] and at 745 and 925 cm^{-1} (symmetric and asymmetric vibrations of a pyranose ring).

Thus, the main polysaccharide of the pectin of sugar-beet leaves is a linear polygalacturonan constructed of α-D-galacturonic acid residues in the pyranose form linked by $1 \rightarrow 4\alpha$ glycosidic bonds.

EXPERIMENTAL METHOD

Isolation of the Pectin. The experiments were performed with leaves of sugar beet of the monogerm variety Yaltushkovskaya, 1972 crop, grown in the Odessa oblast.

The samples were freed from dirt, dried, comminuted, and then analyzed. The soluble and water-insoluble pectins were obtained as described by Tishchenko and Sapozhnikova [3]. A titrimetric method was used for the quantitative determination of the pectin substances and their qualitative characterization [5].

Hydrolysis of the Pectin. The pectin was hydrolyzed with 2N sulfuric acid at 101° C for 6 h. The monosaccharides were identified by comparison with authentic samples on paper chromatography. As the mobile solvent we used pyridine–butanol–water–benzene (3 : 5 : 3 : 1) and as the chromogenic agent aniline phthalate.

Characterization of the Pectin. The molecular weight determined by the viscosimetric method [6] was 1700 c.u., the amount of polyuronide 71.4%, and of ash 7%, the degree of methoxylation 65%, and the proportion of acetyl groups 0.52%. The material was fractionated on DEAE-cellulose treated by Neukom's method [7]. The fractions were monitored by the anthrone method.

Isolation of the Polyuronide. The polyuronide was isolated by the partial acid hydrolysis of the pectin [8]. Yield 41.5%, $[\alpha]_D^{20} + 255°$. Hydrolysis gave only galacturonic acid.

Reduction of the Polyuronide. Since the methylation of the polygalacturonide takes place with great difficulty, it was first methoxylated with diazomethane and reduced with potassium tetrahydroborate to the corresponding galactan [9].

The galactan was methylated three times by Hakomori's method [10]. This gave the fully methylated compound (no hydroxyl absorption band in the IR spectrum).

Hydrolysis of the Methylated Galactan. The methylate product was formalized with 90% HCOOH at 100° C for 1 h and was then hydrolyzed with 0.5 M H_2SO_4 at the same temperature for 14 h. The hydrolyzate was neutralized with $BaCO_3$, centrifuged, and evaporated to small volume, and was then chromatographed on paper. 2,3,6-Tri-O-methylgalactose and 2,3,4,6-tetra-O-methylgalactose were detected. Part of the methylated galactan was subjected to methanolysis with a 4% solution of HCl in absolute methanol followed by GLC.

Periodate Oxidation of the Galactan: Smith Degradation. The galactan was oxidized with a 0.3 M solution of sodium periodate at room temperature for 3 days. The oxidized and dialyzed galactan was reduced with sodium tetrahydroborate. The resulting polyol was hydrolyzed with 0.2 M HCl at room temperature for 6 h. Threitol and glycerol were found among the hydrolysis products by paper chromatography.

SUMMARY

The polygalacturonide of the pectin of sugar-beet leaves has been studied. It has a linear carbohydrate chain consisting of D-galacturonic acid residues in the pyranose form linked by α-(1→4) bonds.

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