STRUCTURE OF A PECTIN HOMOGALACTURONAN

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A homogalacturonan has been isolated in the pure form from industrial apple pectin, and its structure has been studied by ^{13}C NMR spectroscopy. It has been shown that the apple pectin macromolecule consists mainly of linear macromolecules of homogalacturonan and a rhamnogalacturonan.

Pectin is a traditional gelling agent for the production of jams and jellies, and the area of its use extends to the production of fruit, dairy, and dessert products and pharmaceuticals [1].

The basic structure of pectins obtained from various sources has been studied with the use of chemical and enzymatic methods of degradation and has been generalized in a number of reviews [2-6]. At the present time, NMR spectroscopy is an informative method for characterizing the composition and sequence of the polysaccharide units [7-9]; however, because of the complexity of their structures, experimental approaches to the analysis of pectin substances are still at the stage of preliminary development.

The literature contains a small number of papers on the NMR spectroscopy of pectins – in particular, sugar-beet pectin [10], a tobacco rhamnogalacturonan [11], the pectin substances of grapes [12], and others [13, 14]. In these studies the structures of the pectins were established on the basis of a calculation of the spectra of linear and branched polysaccharides by an additive scheme with the aid of a computer.

We have investigated by means of ^{13}C and ^{1}H NMR spectroscopies the structure of a homogalacturonan isolated from apple pectin. Industrial apple pectin was purified by successive treatment with alcohol acidified with hydrochloric acid, centrifugation, and ultrafiltration. The yield of pectin and the number of galacturonic acid residues in it were determined at each stage of purification.

The results, which are given in Table 1, show that there was an increase in the amount of galacturonic acid in the pectin from stage to stage. In the first stage the amount of galacturonan increased because of the extraction of a large amount of alcohol-soluble neutral oligosaccharides and the removal of bivalent metal ions. In addition, because of the high concentration of hydrogen ions, the cleavage of the acid-labile bonds of the arabinans and arabinogalactans present in the side-chains of the pectin macromolecule was possible.

A microgel – a high-molecular-mass cross-linked polymer produced by the degradation of protopectin (with a molecular mass of several million) – was separated off by centrifugation at 7200 g. Although, according to the literature [15], the microgel is eliminated by the ultracentrifugation of a pectin solution, our results showed that the amounts of microgel removed by ultracentrifugation and by centrifugation at 7200 × g were the smae.

In the following stage, it was possible to easily concentrate the partially purified pectin and separate the low-molecular-mass fraction by ultrafiltration. The pectin obtained (PVB-Ch) was separated on an ion-exchange resin – DEAE-cellulose – by stepwise elution with phosphate buffers into four fractions. Figure 1 shows an elution profile of the pectin fractions. The heights of the peaks in the diagram correspond to the proportions of the eluted pectin in relation to the total amount of pectin deposited. The first two pectin fractions (1 and 2) (eluted by 0.05 and 0.15 M phosphate buffers) mainly contained neutral polysaccharides and amounted to 9.04 and 3.07%, respectively, of the amount of pectin deposited.

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TABLE 1. Characteristics of the Pectins before and after Purification

Stage of purification	Yield on the initial sample, %		Amount of galacturonic acid, %
	pectin	ballast subtances	
Initial pectin (PVB)	100	—	50,4
1. Treatment with acidified (HCl) alcohol	70,4	29,60	74,56
2. Centrifugation			
a) 7 thousand rpm	62,0	8,36	81,2
b) 40 thousand rpm	61,8	8,61	81,6
3. Ultrafiltration	57,5	4,55	90,2

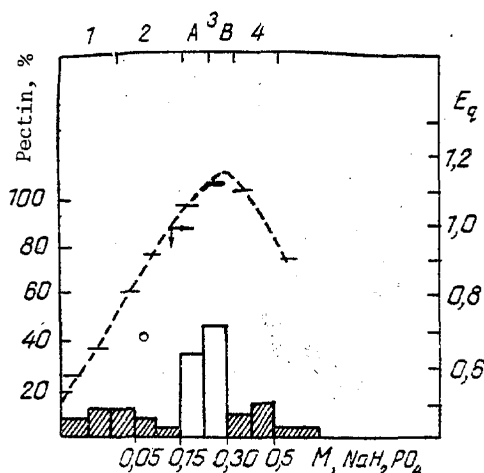


Fig. 1. Profile of the elution of the pectin fractions by phosphate buffers.

The bulk of the pectin constituted the acidic fractions 3A and 3B (Table 2), amounting to 38.9 and 42.1%, respectively. In addition, a small amount (7.82%) of pectin was obtained with the 0.5 M phosphate buffer.

As was to be expected, stepwise elution with larger amounts of buffers but with narrower concentration ranges gave a great deal of information on the fractional distribution profile of the pectin. Figure 1 also gives the dependence of the magnitude $E_q = E_{298}/E_{315}$ on the concentration of phosphate buffer. It follows from the E_q values that the first two fractions (1 and 2) were enriched with neutral polysaccharides. With an increase in ionic strength, this magnitude rose, and in fractions 3A and 3B it reached values close to those for pure galacturonic acid. At the same time, the amount of these fractions was the largest (81%), which indicated the predominance of a homogalacturonan chain in the pectin under investigation.

Certain authors [16, 17] have shown that the separation of pectin on DEAE-cellulose depends on the distribution of ester groups along the galacturonan chain. These results confirm the fact that buffers with higher concentrations are required for the desorption of the less methylated pectins. However, with an increase in the degree of esterification of the pectin chain the charge density rises and the desorption of the pectin macromolecules becomes more difficult. This gives grounds for considering that fractions 3A, 3B, and 4 differed from one another with respect both to the ratio of galacturonic acid and neutral sugars and to the degree of esterification of the carboxy groups.

We made a detailed study of the compositions and structures of fractions 3A and 3B with the aid of ^{1}H and ^{13}C NMR spectroscopies. For comparison we recorded the ^{13}C NMR spectrum of the purified pectin before the stage of ion-exchange chromatography — PVB-Ch.

In the pectin PVB-Ch, in addition to the main signals characteristic for the repeating units of a homogalacturonan, resonances were observed in the regions of 105.8, 79.01, 76.3, 73.9, 70.0, and 18.32 ppm (Table 3) showing the presence of a rhamnogalactouronan at the end

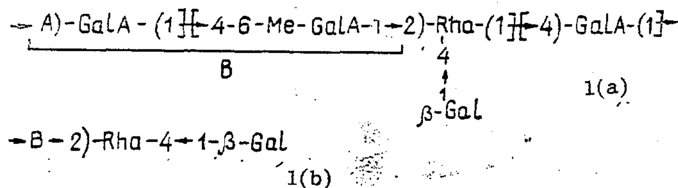
TABLE 2. Characteristics of the Pectin Fractions

Concentration of the phosphate buffer, M	Fraction No.	Symbol	Yield of the fraction	Amount of polysaccharide residues, %	
				galacturonic acid	neutral sugars
0,05	1	PVB-Ch1	9,04	30,0	70,0
0,15	2	PVB-Ch2	3,07	62,5	37,5
0,30	3A	PVB-Ch3A	38,9	90,2	9,8
	3B	PVB-Ch3B	42,1	95,4	4,6
0,50	4	PVB-Ch4	7,82	82,5	7,5

TABLE 3. Details of the ^{13}C NMR Sepctra of Fragments of the Pectin Polysaccharides

Fragments of the pectin polysaccharides	Chemical shifts, ppm					
	C1	C2	C3	C4	C5	C6
(1→4)-α-GalA	100,6	69,8	70,6	80,6	72,3	176,8
6-Me-GalA	101,6	55,9	—	80,8	73,3	172,6
β-*D*-Galp-(1 →	105,8	73,9	—	79,01	76,3	—
β-*D*-Galp-(1→4)-α-Rha(L)	94,1	—	71,2	82,5	70,0	18,32

of the chain or in the side-chains of which there were β-galactopyranosyl residues. This section of the chain can be illustrated schematically in the following way:



In the ^{13}C NMR spectra of the acidic fractions 3A and 3B the resonances characteristic for neutral sugars had disappeared almost completely, except for a weak signal in the 18.32 ppm region. The ^{13}C NMR spectra of fractions 3A and 3B differed only in the intensities of the resonance at 172.6 ppm. At the same time, in the ^{13}C NMR spectra of the purified pectin and of the acidic fractions 3A and 3B obtained from it signals were observed in the 62.4, 75.1, and 79.2 ppm regions which probably related to a starch-like glycan.

In the ^{13}C NMR spectra of fractions 3A and 3B that had been freed from starch (α-amylose), resonances were observed at 69.8, 70.6, 72.3, 80.6, 100.6, and 176.8 ppm, which agree well with previous results [14] for the residues of a homogalacturonan from flax pectin. ^{13}C resonances at 55.9, 73.3, 80.8, 101.6, and 172.6 ppm were assigned to methylated galacturonic acid residues, since the saponification of the pectin led to the disappearance of these signals. In the ^{1}H NMR spectra, likewise, signals were detected for protons in the H(1−5) positions at 5.05, 3.82, 4.04, 4.44, and 4.78 ppm, respectively. According to the literature [16, 17], these resonances are characteristic for a pure →4)-α-D-GalA-(1-homogalacturonan). A weak signal in the 18.32 ppm region showed the presence of terminal →4-α-Rha(L) residues.

Thus, on the basis of the results described and those of previous work [18] it may be concluded that apple pectin is a mixture of the linear plymers homogalacturonan and rhamnogalactoruronan with branched polysaccharides the macromolecules of which include galacturonic acid residues.

EXPERIMENTAL

Industrial apple pectin (Bendery factory, Moldova) was treated with a mixture of 70% ethanol, concentrated HCl, and water in a ratio of 14:3.6:2.4, respectively, at room temperature for 24 h. After this, it was washed with 70% alcohol until the reaction for Cl^- ions was negative, and then with 96% alcohol and with acetone and was dried. To eliminate microgel, a 0.5% solution of the pectin as centrifuged at 7 thousand rpm for 1.5 h. The low-molecular-mass fraction was eliminated from the pectin solution with the aid of an ultrafiltration membrane. The pectin was precipitated from the solution with alcohol and was dried.

For chromatography we used DEAE-52 (Serva). The ion-exchange chromatography of the pectin substances was conducted by the procedure of [19]: 434 mg of pectin was dissolved in

40 ml of 0.025 M NaH_2PO_4, pH 5.1, and the solution was passed through a column (1.56 × 30 cm) of DEAE-cellulose. Separation was achieved by stepwise elution with phosphate buffers having concentrations of 0.025, 0.05, 0.15, 0.3, and 0.5 M. The fractions were desalted on a UPM-50 ultrafiltration membrane. The ratio of galacturonic acid and neutral sugars was determined as in [20]. For this, 5 ml of H_2SO_4 (conc.) was added to 0.5 ml of an eluate, and the UV spectrum was recorded on a Hitachi spectrometer (Japan) in the 340-280 nm region against a comparison solution (0.5 ml of H_2O in 5 ml of concentrated H_2SO_4).

As standards we used galacturonic acid from Serva (FRG) and glucose from Leidhold. Galacturonic acid was also determined by the carbazole method [21]. The ^{13}C and ^{1}H NMR spectra of solutions of the pectins in D_2O were taken on a Bruker WP-80DS instrument at 80°C in the accumulation regime. Dimethyl sulfoxide was used as internal standard.

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