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PECTIN SUBSTANCES OF *Amaranthus cruentus*

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Pectin substances have been isolated from the epigeal part of love-lies-bleeding, Amaranthus cruentus (A. caudatus), *and have been characterized. The presence in them of galacturonic acid, galactose, rhamnose, xylose, arabinose, fructose, and glucose residues has been established. The titrimetric indices of the substances isolated have been determined and they have been studied by IR spectroscopy.*

We have continued an investigation of the chemical composition of plants of the genus *Amaranthus* and of methods for their practical utilization [1].

Amaranths are known as protein-rich crops the introduction which is being intensively pursued both in the New World [2] and in Europe and the countries of Southeast Asia [3]. It has recently been shown that the plant *A. cruentus* L. and some others may be a source for the industrial production of rutin [4] and other substance of practical value, especially pectin substances (PcSs).

According to the literature, of the polysaccharides of plants of the genus *Amaranthus* only the seed starch has been studied [5, 6]. We have isolated the pectin substances from the epigeal part of *A. cruentus* and have studied some of their physicochemical properties. The pectin substances isolated consisted of amorphous cream-colored powders with various tinges and, depending on the method of isolation, readily soluble in water with the formation of viscous solutions. The molecular masses of the samples obtained, determined viscometrically [7], ranged from 30,000 to 130,000 c.u.

The molecular mass was governed both by the pH of the hydrolyzing solution and by the time of hydrolysis, as in [6], and also by other conditions of isolation, including the nature of the hydrolyzing reagent: an inorganic or organic acid or an enzyme. The main functional groups and physicochemical characteristics of the PcSs isolated by the method of [6] in our modification, as determined by titrimetric and other methods [7], are given in Table 1.

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As can be seen from Table 1, the PcSs isolated were of the highly esterified type. Their qualitative monosaccharide composition was represented by galacturonic acid, galactose, rhamnose, xylose, arabinose, fructose, and glucose (Table 2).

The development of the best methods for isolating the pectin substances and further progress in their investigation is being determined by the use of fine and selective methods of analysis. Much information on the structure of pectin substances is given by their IR spectra.

We investigated the IR spectra of the pectin substances at various stages of their isolation. The IR spectra of the pectin substances from love-lies-bleeding are similar to those of apple pectins. Three groups of bands stand out in the 400-2000 cm^{-1} region. The first group is due to the stretching vibrations of carbonyl groups present in different environments. From the ratio

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TABLE 1. Physicochemical Characteristics of the Pectin Substances of *A. cruentus*

Index	Quantitative characteristic
Yield, %	3.7–4.0
Methoxy groups, %	6.6
Acetyl groups, %	0.5
Free carboxy groups, %	8.6
Degree of esterification, %	75.0
Uronide component, %	
via calcium pectate	80.0
by the titration method	82.0
Equivalent weight вес	520
Static exchange capacity, meq/g	
for calcium chloride	10.3
for sodium hydroxide	3.2
Mol. mass, c.u.	30000–130000

TABLE 2. Qualitative and Quantitative Monosaccharide Compositions of the PcSs of *A. cruentus*

Monosaccharide	Quantitative ratio
Galacturonic acid	67.1
Rhamnose	4.1
Xylose	2.1
Arabinose	6.6
Fructose	4.1
Glucose	8.3
Galactose	7.7

of the intensities of the bands in this region it is possible to determine the quantitative characteristics of a pectin: the degree of esterification and the number of free — including ionized — carboxy groups. The mean degree of esterification of the pectins isolated was 75%. Free carboxy groups amounted to about 9%.

The second group of bands is located in the 1200-1500 cm^{-1} region and is due to the C–H deformation vibrations of methyl groups and of pyranose rings and to COH fragments. The nature of the distribution of the intensities in this group shows the presence of structural differences in the love-lies-bleeding pectins as compared with apple pectins.

The most intense, third, group of bands is present in the 1000-1200 cm^{-1} region and relates to the stretching C–C and C–O vibration of pyranose rings. The band contour of this group, together with the results that we obtained on the monosaccharide composition of the pectins isolated, permit the conclusion that this region is not very informative for spectroscopic correlations.

IR spectroscopy proved to be extremely informative in monitoring the purity of pectin at various stages of its isolation. It enables one to check not only impurities of protein nature but also the presence of the calcium salts formed in the hydrolysis of protopectin.

In the preparation of the pectin samples for recording their spectra, the hydrogen of carboxy groups is replaced by potassium ions, which leads to a doubling of some bands in the spectra and to a decrease in the intensity of the 1740 cm^{-1} band.

EXPERIMENTAL

Thin-layer chromatography was conducted on Silufol UV-254 plates in the butan-1-ol–acetic acid–water (8:3:2) system. The spots of the monosaccharides were revealed with aniline hydrogen phthalate. Liquid chromatography was conducted on a chromatograph of the LP series (Laboratorni Pristoje, Prague) under the following conditions: column with Silasorb-NH_2 (4 × 300 m); mobile phase: acetonitrile–water (85:15); detection: refractometric.

IR spectra were taken on a Bruker IRS-113 Fourier IR spectrometer with a resolution of 1 cm^{-1} in the range of 400-4000 cm^{-1}. The PcS samples were ground and mixed with an optically pure powder of dry potassium bromide, and the mixture was tableted in a special mold under a pressure of ~10 t/cm^2.

For the determination of molecular masses, an Ostwald viscometer with a diameter of 0.73 mm was used. The main functional characteristics were determined by known methods [7].

Isolation of the Pectin Substances. The dried herbage of love-lies-bleeding was extracted with a 1% solution of oxalic acid (1:10) at 90°C for 2 h and was filtered off, and the polysaccharides were concentrated by passing the extract through a membrane filter with a pore diamter of 50 kD [sic] in a UFL-1 ultrafiltration apparatus. In the part that had not passed through the membrane, the PcSs were precipitated by the addition of acetone in a ratio of 1:3. The resulting precipitate was filtered off and dried.

Determination of the Monosaccharide Composition. A weighed sample of PcS was hydrolyzed with 2 N H_2SO_4 at 100°C for 14 h. The hydrolysate was neutralized with barium carbonate, filtered, and chromatographed on Silufol UV-254 plates.

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