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POLYSACCHARIDES OF *Cardaria repens* AND A STUDY OF THEIR IR SPECTRA

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Five fractions have been obtained by the fractional precipitation of the water-soluble polysaccharide with alcohol: water-insoluble (fractions 1 and 2) and water-soluble (fractions 3-5). Their Fourier IR spectra have been studied and, on the basis of the results obtained, it is suggested that the water solubility of the fractions examined is connected with strong hydrogen bonds.

Cardaria repens, fam. Brassicaceae (Cruciferae), is a perennial herbaceous plant widely distributed in Central Asia [1]. There have been reports [2] of the presence in this plant of low-molecular-mass compounds: alkaloids, glycosides, flavonoids, coumarins, and vitamins. Carbohydrates have not been studied.

By extracting the epigeal part of *Cardaria repens* with water we isolated a water-soluble polysaccharide (WSPS) with a yield of 8.4%. It consisted of a white powder wih a creamy tinge which, on being redissolved in water, formed a slightly turbid solution with 2.3% of water-insoluble polysaccharide (WISPS). Complete acid hydrolysis and identification of the monosaccharide composition by PC and GLC showed that the WSPS was represented by residues of rhamnose, arabinose, xylose, glucose, and galactose in a ratio of 1.5:13.3:1.0:1.0:21.0, respectively, and uronic acids.

Fractional precipitation with alcohol gave five fractions. A study of their monosaccharide compositions by PC and GLC (in the form of aldononitrile acetates) showed that arabinose and galactose were predominant in all the fractions (Table 1). The fractions obtained were soluble in water. Fractions 3 and 4 formed clear solutions, and this enabled us to use sedimentation analysis [3] to establish for them molecular masses of 63,000 and 56,000, respectively. The partially water-soluble fractions 1 and 2 contained the largest amount of methoxy groups — 2.5 and 3.6% —, the water-soluble fractions 3 and 4 containing 1.3 and 0.7%, respectively.

In order to find the reasons for the water insolubility of some of the polysaccharides studied, we examined the Fourier IR spectra of the WSPS, the WISPS, and fractions 1-5. Their frequency characteristics are given in Table 2.

The IR spectra of fractions 1 and 2 and of the WISPS differed substantially from those of fractions 3-5 in the 600-750, 1000-1070, and 3000-3500 cm^{-1} regions, the greatest differences in the frequency values being characteristic for the first two regions. Fractions 1 and 2 and the WISPS had a broad maximum at 602-655 cm^{-1} and intense absorption at about 1095 and 3390 cm^{-1} while in the water-soluble fractions these were displaced in the high-frequency direction, having values of 620, 1115, and 3420 cm^{-1}, respectively. A feature of the IR spectra of fractions 4 and 5 was the presence of a sharp intense maximum at about 620 cm^{-1} not present for the insoluble fractions 1 and 2.

According to generalizations [4, 5] made from the IR spectra of associated molecules, the stretching C—O vibration falling into the 1000-1070 cm^{-1} region are shifted in the low-frequency direction when hydrogen bonds (HBs) are formed: overtones in the 600-700 cm^{-1} region correspond to O—H···O stretching and deformation vibrations, which have low frequencies of the order of ≤ 200 cm^{-1}; O—H out-of-plane twisting vibrations absorb below 800 cm^{-1}.

On the basis of the facts given above, it may be assumed that the appearance of a broad maximum at 602 cm^{-1} and the shift of ν_{OH} and ν_{C-O} in the low-frequency direction in fractions 1 and 2 with high contents of O—CH_3 groups relative to fraction 4 indicate the formation of OH···OCH_3 HBs, while the appearance of a narrow high-frequency maximum at 612 cm^{-1} and the increase in the ν_{C-O} and ν_{O-H} frequencies suggest weaker HBs between the functional groups of the molecules of fraction 4 and the initial WSPS.

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TABLE 1. Monosaccharide Composition of the WSPS Fractions

Fraction	Yield, %	Number of carbohydrate residues						
		Rha	Ara	Xyl	Glc	Gal	GalUA	GlcUA
1	0.43	1.0	2.0	Tr.	Tr.	2.0	–	+
2	13.2	1.0	8.3	Tr.	Tr.	20.3	–	+
3	20.0	1.0	5.4	Tr.	Tr.	12.0	+	–
4	7.3	1.0	7.3	Tr.	Tr.	17.9	+	+
5	36.8	1.1	5.3	1.0	4.2	7.4	+	+

GAlUA and GlcUA are identified with PC.

TABLE 2. IR Spectra of the WSPS, Fractions 1-5, and the WISPS, cm^{-1}

Fraction	ν_{OH}	ν_{C-O}	δ_{O-H}
	Water-insoluble		
WISPS	3382	1092	555
1	3387	1096	602
2	3387	1096	602
	Water-soluble		
WSPS	3394	1122	619
4	3424	1114	518
5	3409	1111	618
3	3392	1196	
		1126	658
		1095	602

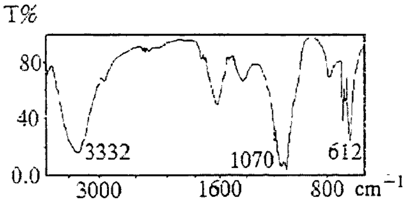

Fig. 1. IR spectrum of fraction 3.

The appearance of a fine vibrational structure of the absorption bands in the 600-700 and 1000-1070 cm^{-1} regions in fraction 3 is apparently explained by the vibrational interaction of neighboring functional groups, weakening the HBs in this polysaccharide.

Thus, the spectral characteristics obtained permit the assumption that the solubility of fractions 1-5 is connected with the strength of the HBs.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40±5°C. Monosaccharides were chromatographed on FN 3, 11, 12 paper (Germany) by the descending method in the system (by volume) butan-1-ol—pyridine—water (6:4:3), the revealing agent being acid aniline phthalate.

The GLC of the samples was conducted on a Chrom-5 instrument with a flame-ionization detector, steel column (200 ± 0.3 cm) filled with 5% XE-60 on Chromaton N-AW 0.200-0.250 mm, carrier gas helium (60 ml/min), 210°C.

Samples of the WSPS and their fractions were hydrolyzed with 2 N H_2SO_4 at 100°C for 8 h. Aldononitriles were obtained as in [6].

Ultracentrifugation was conducted on a MOM-3170 instrument (50,000 rpm) at 20°C, rate of scanning 30 min. The fractions were studied as 1% aqueous solutions.

The IR spectra of the samples were taken on a Perkin-Elmer model 2000 Fourier IR spectrometer in tablets molded with KBr. Number of scans 100.

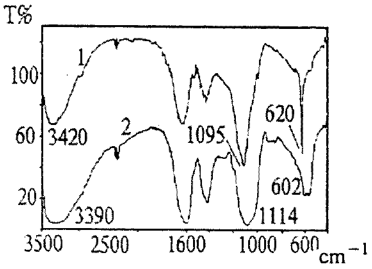

Fig. 2. Comparison of the IR spectra of fraction 4 (**1**) and of the WISPS (**2**).

Isolation of the WSPS. The comminuted raw material (500 g) was extracted successively with chloroform, methanol, and 86% alcohol with boiling on the water bath for 1 h. The WSPS was extracted by water at room temperature from the residue with a yield of 42.39 g.

Fractional Precipitation with Alcohol. The dissolution of 10 g of WSPS in 250 ml of water formed a turbid solution. This was centrifuged, and the deposit was separated off, washed with alcohol, and dried in vacuum over P_2O_5. The yield of water-insoluble polysaccharide was 0.23 g.

With constant stirring, 125 ml of alcohol was added to 250 ml of the supernatant. The precipitate that deposited was separated off, washed with alcohol and acetone, and dried in vacuum over P_2O_5. The yield of fraction 1 was 0.43 g. Then 125 ml of alcohol was added to the supernatant and the precipitate that formed was treated in the same way as before. The yield of fraction 2 was 1.32 g. Fractions 3 and 4 were obtained by adding 250 ml of alcohol to the supernatant in each case. The yields of fractions 3 and 4 were 2.0 and 0.73 g, respectively. The supernatant alcoholic solution was evaporated to the state of a syrup and was precipitated with alcohol. The yield of fractions was 3.68 g.

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