CHARACTERISTICS OF THE WATER-SOLUBLE POLYSACCHARIDES OF THE LEAVES OF *Silphium perfoliatum*

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The characteristics of the water-soluble complex of the leaves of *Silphium perfoliatum* are given. The presence in them of acidic components of the type of pectic substances containing considerable amounts of compounds of protein nature has been shown. It has proved to be possible to separate the latter from the carbohydrate component by fractionation on DEAE-cellulose.

The primary structures of the polysaccharides of a number of herbs have been investigated previously [1]. We give the characteristic features of the individual components of the water-soluble complex (WSC) of the leaves of *Silphium perfoliatum* (cup rosinweed).

The raw material was fixed by heat-drying, comminuted, and defatted. To eliminate the free mono- and oligosaccharides the residue was treated with 82% ethanol. The WSC was extracted with hot water and the solution was filtered. The filtrate was concentrated in vacuum. This led to the formation of a precipitate (fraction 1). The action of different volumes of ethanol on the solution led to two more fractions: 2 and 3. The characteristics of the fractions were as follows:

	1	2	3
Amount in the raw material, %	2.8	1.8	8.3
Nitrogen, %	5.6	2.5	1.9
Polysaccharides, %	30.0	50.0	43.0
Composition of the polysaccharides (molar ratios)			
Uronic acids	4.0	4.0	3.4
Galactose	0.5	0.3	1.0
Glucose	0.9	1.5	0.9
Arabinose	1.0	0.4	1.2
Xylose	0.8	0.4	—
Ribose	0.5	Tr.	—
Rhamnose	0.5	Tr.	—

The water-soluble polysaccharides of the rosinweed leaves include the set of monosaccharide residues that is on the whole characteristic for a plant raw material. The glucose found was not connected with the presence of starch, since the iodine test and the action of amylase did not give positive results.

The presence of a large amount of uronic acids shows that the WSC contains an acidic component of the type of pectic substances.

All these fractions differed in their quantitative and qualitative monosaccharide compositions. Substances 2 and 3 were fractionated additionally, using Sephadexes of various types. Because of its poor solubility, fraction 1 was not studied. The use of Sephadex G-100 gave two curves (carbohydrate and protein). The maximum intensity of the carbohydrate peak did not coincide with the maximum intensity of the protein peak. The fractionation of the same complex on Sephadex G-200 enabled us to separate the carbohydrate component into two fractions with different molecular weights (2a, 2b) and in one of them — the one of higher molecular weight — the protein component was practically absent. Protein was present in

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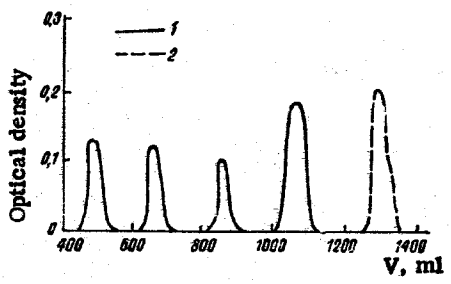

Fig. 1

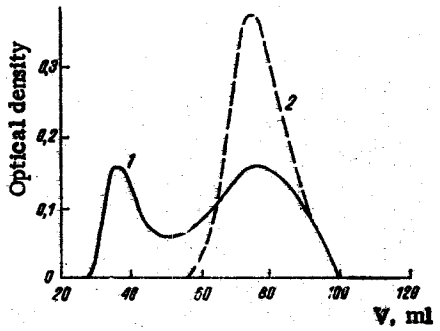

Fig. 2

Fig. 1. Elution curve of the fractionation of polysaccharide 2 on DEAE-cellulose: 0-200 ml) water; 200-400 ml) 0.1 M NaH_2PO_4; 400-600 ml) 0.3 M NaH_2PO_4; 600-800 ml) 0.5 M NaH_2PO_4; 800-1000 ml) 0.1 N NaOH; 1000-1200 ml) 0.3 N NaOH; 1200-1400 ml) 0.5 N NaOH; 1) carbohydrate; 2) protein.

Fig. 2. Elution curve of the fractionation of polysaccharide 3 on Sephadex G-100: 1) carbohydrate; 2) protein.

the second component, but the maxima of their peaks did not coincide, which indicates a weak bond between these polymers.

Analysis of the monomeric composition of the two fractions showed that they differed only slightly and were practically pure polyuronides:

Fraction	Uronic acids	Galactose	Glucose	Arabinose	Xylose	Rhamnose
2a	5.0	1.0	0.1	0.1	Tr.	Tr.
2b	4.0	0.8	Tr.	Tr.	Tr.	—

Fraction 2 was completely precipitated by Cetavlon and aluminum sulfate, which also confirmed the absence of neutral polysaccharides from it. When it was fractionated on DEAE-cellulose, four polysaccharide fractions containing no protein impurity were obtained. The protein component was eluted separately with 0.5 M NaOH after the polysaccharide fraction (Fig. 1). The monosaccharide compositions of the hydrolysates of the fractions are given below (molar ratios):

Fraction	Galactose	Glucose	Arabinose	Xylose	Uronic acids
2c	1.0	1.0	1.0	0.1	3.0
2d	1.0	Tr.	1.0	0.2	3.0
2e	1.1	1.0	1.0	0.2	4.2
2f	0.8	Tr.	0.6	Tr.	5.0

According to the results of fractionation, polysaccharide 2 was inhomogeneous and consisted of a set of acidic products differing by their uronic acid contents and by the ratio of the neutral monomers. There were no neutral polysaccharide components. The possibility of the separation of the protein component showed the absence of a covalent bond between the polysaccharide and the protein.

The natures of the elution curves for the gel filtration of polysaccharide 3 on Sephadexes G-100 (Fig. 2) and G-200 were identical. Both curves were characterized by the presence of two carbohydrate peaks, the second of which, corresponding to the fraction of lower molecular weight, was bound to protein. The monomeric compositions of the fractions obtained in the gel filtration of the polysaccharide 3 were as follows (molar ratios):

Fraction	Uronic acids	Galactose	Glucose	Arabinose	Xylose
3a	2.0	1.0	1.0	0.4	Tr.
3b	3.3	0.5	0.4	1.0	Tr.

The fractions, which had the same qualitative composition, differed only by the molar ratios of the monosaccharide residues.

The fractionation of product 3 on DEAE-cellulose gave three polysaccharide fractions the monomeric compositions of which were as follows (molar ratios):

Fraction	Uronic acids	Galactose	Glucose	Arabinose
3c	4.0	0.8	0.2	1.0
3d	3.0	1.3	0.3	1.0
3e	3.0	0.4	0.3	1.0

As in the case of product 2, the protein component was eluted separately with 0.5 M NaOH.

EXPERIMENTAL

The cup rosinweed leaves were fixed by hot drying, comminuted, and defatted with a mixture of methanol and benzene (1:3). The residue was extracted with 82% ethanol to eliminate free sugars.

The water-soluble complex was isolated by extraction with hot water (75-80°C) with continuous stirring exhaustively — until the anthrone reaction for carbohydrates was negative.

Fractionation of the Complex. The extract obtained (3500 ml) was concentrated in vacuum to 150 ml. This led to the deposition of a precipitate — fraction 1. The addition of 1 and 15 volumes of alcohol to the centrifugate gave fractions 2 and 3.

Characterization of the Fractions. The individual fractions were characterized by the amount of them in the raw material and by their nitrogenous substances [2] and carbohydrate compositions [3], and they were fractionated on Sephadexes G-100 and G-200 and DEAE-cellulose [4].

SUMMARY

The wild herb Silphium perfoliatum contains a complex of water-soluble polysaccharides characterized by the presence of protein components and differing by their ratios of monosaccharide residues in the polysaccharides.

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