CARBOHYDRATES OF Eremurus regeli

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UDC 547.917

We have investigated the carbohydrate complex of the epigeal and hypogeal organs of Eremurus regeli Vved. [1-5] and the dynamics of its accumulation as a function of the vegetation period of the plant.

The plants were collected from a single site close to the village of Kaplanbek, South Kazakhstan oblast, during 1972 in the following periods of development: regrowth of the epigeal part (March 30), formation of peduncle (April 20), full flowering with unripe fruit (May 26), ripening of the seeds (leaves shrivelled, June 21), and after the withering of the epigeal part (July-November). In each organ of the plant we determined the amount of reducing substances (RSs) before and after inversion, the water-soluble polysaccharides, the pectin substances (PSs), the hemicelluloses A and B, and the α-cellulose (Table 1).

The table shows that in the leaves the amount of RSs gradually increases up to the period of formation of the peduncle and then falls. In the tuber roots the amount of RSs reaches a maximum in the period of intensive growth, then gradually falls, and then rises again in the period of deep dormancy.

In the epigeal and hypogeal organs of the plants, polysaccharides are found in the earliest phases of development and undergo quantitative changes throughout the vegetation period.

In the quantitative respect, water-soluble polysaccharides (eremuran) predominate in the tuber roots and PSs in the leaves. The amount of eremuran reaches a maximum in the tuber roots collected in August, and then falls because of the partial decomposition of the polysaccharide, as is shown by an increase in the amount of RSs. The pectin substances are distributed dissimilarly in the different organs of the plant. In the epigeal parts, in all periods of development of the plant, the amount of PSs is correspondingly greater than in the tuber roots. Attention is attracted by the relationship between the amount of PSs and the amount of α-cellulose – a rise in the amount of one of these substances is always accompanied by a fall in that of the other, and conversely.

We have also begun a study of the structure of the pectin substances. An isolated sample of the PSs [6] was subjected to complete acid hydrolysis, and in the acid hydrolyzate arabinose, xylose, and rhamnose were detected chromatographically. Galacturonic acid, obtained via its barium salt, was identified by comparing the R_f values and IR spectra and by the determination of a melting point of a mixture with an authentic sample of the acid.

Saponification of the initial PSs led to pectic acid with a yield of 86.5%, and when this was subjected to complete acid hydrolysis the same monosaccharides and galacturonic acid were detected chromatographically. The amount of galacturonic anhydride in the PSs was determined by decarboxylation with 19% hydrochloric acid [7]. This showed the presence of 65.7% of galacturonic anhydride. Consequently, the main components of the PSs are galacturonic acid, arabinose, xylose, and rhamnose; galactose was found in very small amounts.

EXPERIMENTAL

The freshly collected plant material fixed with boiling 96% ethanol (1:10) was used for analysis. An individual weighed portion of the raw material was dried at 100-105°C to constant weight, and the absolutely dry weight was determined. Solutions were evaporated in a rotary evaporator at 40°C. Chromatography

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 429-432, July-August, 1974. Original article submitted April 10, 1973.

TABLE 1. Dynamics of the Accumulation of the Carbohydrates of Eremurus regeli (% on the absolutely dry weight)

Organs of the plant and time of collection	RSs		Polysaccharides					Total carbohydrates
	before inversion	after inversion	water-soluble	RSs	hemicellulose A	hemicellulose B	α-cellulose	
Leaves								
30.III+	14,6	11,9	Traces	5,4	11,6	8,5	3,3	55,3
30.III++	17,0	10,6		7,3	18,4	3,4	3,6	60,3
20.IV	25,6	15,7	1,9	4,3	12,7	3,9	4,2	68,3
26.V	2,2	27,3	3,5	9,4	16,3	3,7	6,7	69,1
21.VI	5,5	1,8	1,5	17,1	4,2	6,8	12,2	49,1
Peduncle								
20.IV	24,6	21,2	Traces	2,5	5,2	14,3	6,4	74,2
26.V	6,9	3,6	0,5	1,8	6,9	7,9	25,9	53,4
21.VI	0,2	0,2	—	3,2	6,9	5,2	29,5	45,2
Fruit								
26.V*	0,9	0,5	1,7	3,3	4,7	3,8	6,7	21,6
21.VI†	0,3	0,6	1,7	4,6	15,2	7,0	8,1	37,5
Tuber roots								
30.III+	13,0	6,2	0,9	2,2	9,4	2,1	1,5	35,3
30.III++	29,6	8,5	2,4	4,8	7,1	2,4	4,2	59,0
20.IV	21,0	27,5	7,9	3,4	1,3	6,9	0,1	68,1
26.V	2,5	27,6	20,9	3,2	10,4	3,2	3,8	71,5
21.VI	0,7	23,2	24,3	2,6	4,1	4,5	10,2	69,5
31.VII	3,4	16,9	23,8	2,6	1,4	3,6	2,5	54,2
30.VIII	1,0	18,8	27,9	2,7	5,2	9,4	5,1	70,1
30.IX	1,8	22,5	19,0	4,3	10,9	0,8	3,9	63,2
30.X	2,6	22,0	12,4	2,4	0,8	4,2	3,3	47,7
24.XI	7,9	36,5	10,2	2,2	0,5	4,6	7,7	66,6

Note. + – length of the leaves 5 cm; ++ – 15 cm.
* – tip of the peduncle with unripe fruit;
† – with ripe fruit.

was performed on FN-1 paper (Czechoslovakia) by the descending method and TLC on silica gel impregnated with 0.3 M NaH_2PO_4 in the following systems: 1) butan-1-ol–acetic acid–water (4 : 1 : 5) and 2) butan-1-ol–ethanol–0.1 N HCl (1 : 10 : 5); the spots were revealed with aniline phthalate.

The carbohydrates were isolated successively from a single sample of the raw material. First, the free sugars were extracted four times with 82% ethanol [8], and then the water-soluble polysaccharides, the pectin substances, the hemicelluloses A and B [9], and the α-cellulose were isolated.

Isolation of the Pectin Substances. The free sugars and water-soluble polysaccharides were removed from 72.5 g of raw material, and the residue was extracted twice with a mixture of equal volumes of 0.5% solutions of oxalic acid and ammonium oxalate (1 : 70) at 70°C. This gave 13.15 g of a light-brown powder of the initial PSs with an ash content of 2.24%.

Purification of the Pectin Substances. This was done by adding ethanol to an aqueous solution of the initial PSs. They formed an odorless cream-colored fibrous powder soluble in water (2% at 50-60°C). Yield 11.46 g. A mixture of 2 g of the purified PSs with 100 g of sugar formed a jelly of good strength.

Demineralization of the Pectin Substances. A solution of 0.5 g of the initial PSs in 100 ml of water was treated with the ion-exchange resins KU-1 (H^+) and AN–2F (OH^-) for 24 h each. Then the solution was precipitated with ethanol (1 : 3), and the precipitate was dried in a vacuum desiccator (P_2O_5). Yield 0.18 g. Ash content 1.97%.

Hydrolysis of the Pectin Substances. Weighed amounts of the three samples (initial, purified, and demineralized PSs) were heated with 1 N H_2SO_4 in sealed tubes in the boiling water bath for 24 h. The hydrolyzates were neutralized with $BaCO_3$, and the solutions were separated from the precipitate, evaporated, and chromatographed in system 1. All three hydrolyzates were found to contain the same sugars: rhamnose, arabinose, xylose, galacturonic acid, and traces of galactose. The same monosaccharides were found on TLC in system 2.

Galacturonic Acid. The initial PSs (0.5 g) were hydrolyzed with 25 ml of 1 N H_2SO_4, the reaction mixture was neutralized with $BaCO_3$ and filtered, and the filtrate was evaporated to 5 ml and treated with

20 ml of ethanol. The precipitate that deposited was separated off (0.2 g) and hydrolyzed with 10 ml of 1 N H_2SO_4 in the boiling water bath for 14 h. The hydrolyzate was neutralized with $BaCO_3$, the filtrate was treated with ethanol, and the precipitate formed was purified by reprecipitation and was dried. Yield 0.1 g. The precipitate was dissolved in 10 ml of water and treated under dynamic conditions with KU-2 cation-exchange resin (H^+). The filtrate was evaporated to dryness, and the residue was dissolved in ethanol. The precipitate that deposited was recrystallized from water, mp 134-135°C, R_f 0.18 (TLC in system 2).

IR spectrum: ν_{max} 3600-3200 (OH^-), 1730, 1640 cm^{-1}.

Pectic Acid. A solution of 4.0 g of the initial PSs in 120 ml of water was treated with 20 ml of 1 N NaOH, the mixture was stirred at room temperature for 30 min, and then 30 ml of 1 N HCl was added. The precipitate formed was separated off, washed with water and ethanol, and dried at 50-60°C. Yield 3.46 g.

IR spectrum: ν_{max} 3400, 1735, 1630 cm^{-1}.

The hydrolysis of the pectic acid was performed with 1 N H_2SO_4 for 6 h. The hydrolyzate was chromatographed on paper in system 1 and in a thin layer of silica gel in system 2. Xylose, arabinose, rhamnose, galactose, and galacturonic acid were found.

SUMMARY

1. In a study of the carbohydrates of Eremurus regeli Vved., according to the period of development of the plant, it has been found that in the leaves the maximum amount of pectin substances is present in the period of the ripening of the seeds. In the tuber roots, water-soluble polysaccharides form the main component and they are present in maximum amount after the withering of the epigeal part.

2. A preliminary investigation of the pectin substances from the leaves has shown that they belong to the heteropolysaccharides consisting of galacturonic acid and the neutral monosaccharides xylose, rhamnose, arabinose, and galactose.

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