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POLYSACCHARIDES OF *Eremurus*.

X. CHARACTERISTICS OF THE POLYSACCHARIDES OF *Eremurus lactiflorus* AND *E. Luteus*

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Water-soluble polysaccharides have been isolated from two species of *Eremurus* — *E. lactiflorus* and *E. luteus* — with yields of 13.5% and 20.5%, respectively. They contained mainly glucose and mannose in ratios of 1:5 and 1:3.1. The polysaccharides of *E. lactiflorus* were separated from a column of DEAE-cellulose. The yield of neutral fraction was 10.3%. Gel filtration of the polysaccharides on Sephadex G-200 showed their polydispersity. Homogeneous fractions were obtained by fractional precipitation with ethanol. They have been characterized with respect to monosaccharide composition, molecular weight, and IR spectra.

It has been shown previously [1, 2] that the tuberous roots of *Eremurus* are rich in water-soluble polysaccharides. We have isolated the polysaccharides (PSs) by the method of Stepanenko et al. [3] and have freed them from protein substances as described by Sevag [4]. From *E. lactiflorus* O. Fed. we isolated 13.5% of PSs (A), and in a hydrolysate of these by paper chromatography (PC) we detected arabinose, galactose, mannose, glucose, and uronic acids. The ratio of glucose and mannose according to gas–liquid chromatography (GLC) was 1:5. The amount of O-acetyl groups was 4.1% [5]. From *E. luteus* Bak. we isolated 20.5% of PS (B) consisting of glucose and mannose in a ratio of 1:3.1 and containing 9.1% of O-acetyl groups. In order to separate it from acidic PSs, polysaccharide A was passed through a column of DEAE-cellulose in the acetate form. Water eluted a neutral fraction (A-1) with a yield of 10.3% (on the air-dry raw material) (Scheme 1).

The gel filtration of polysaccharides A-1 and B on a column of Sephadex G-200 showed their polydispersity (Fig. 1). Consequently, the polysaccharides were mixtures of polymer homologs.

To obtain homogeneous PCs they were subjected to fractional precipitation with ethanol from aqueous solutions [6]. On fractionation, both PCs gave several fractions: the first fraction from A-1 with a yield of 58% proved to be homogeneous (A-2) (Fig. 2); B gave a second fraction (B-1) with a yield of 60%. Hydrolysates of A-2 and B-1 were found to contain glucose and mannose in ratios of 1:2.8 and 1:3.4, respectively.

Fraction A-2 consisted of a white amorphous powder soluble in water with η_{rel} = 20.5 $[\alpha]_D^{20}$ −21.7° (c 0.736; H_2O). The IR spectrum of A-2 contained absorption bands at (cm^{-1}) 3600-3200 (OH), 1730 and 1250 (ester group), 880 (β-glycosidic bond) and 815 (hexapyranose ring) [7]. A quantitative determination [5] showed the presence of 2.05% of O-acetyl groups.

Polysaccharide B-1 formed a cream-colored powder, a 1% aqueous solution of which formed a viscous colloidal system with η_{rel} = 9.66. The amount of O-acetyl groups was 7.4%. Its IR spectrum was similar to that of A-2, i.e., it had the same absorption bands.

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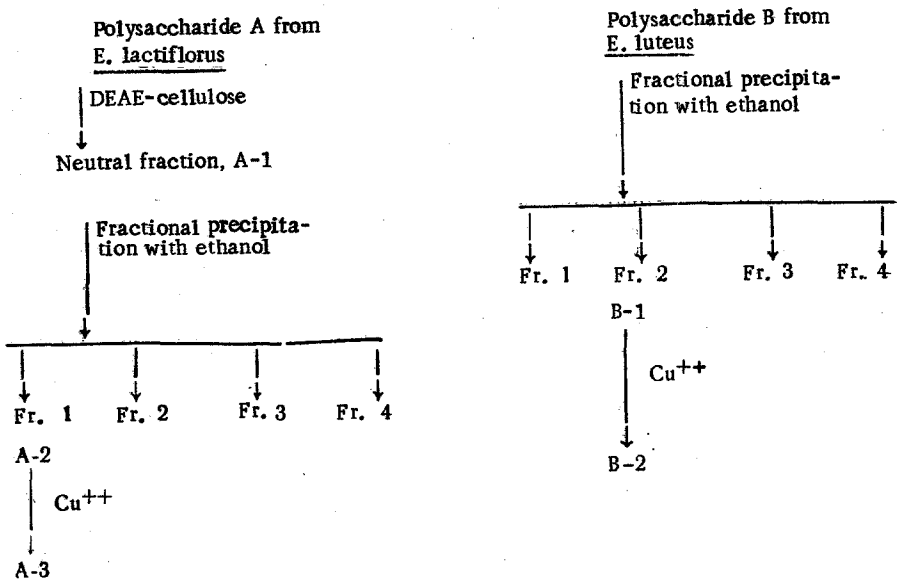

Scheme 1. Separation of the polysaccharides.

The ultracentrifugation of polysaccharides A-2 and B-1 (Fig. 2) showed one peak in each case, indicating their homogeneity.

On treatment with Fehling's reagent [8], polysaccharides A-2 and B-1 formed copper complexes the decomposition of which yielded A-3, with a glucose—mannose ratio of 1:2.7, and B-2, with a ratio of 1:3.6, respectively. The IR spectra of the products A-3 and B-2 obtained differed from the spectra of A-2 and B-1 by the absence of the absorption band of an ester group. Consequently, in their treatment with Fehling's reagent they underwent deacetylation.

The retention of the ratios of the monosaccharides in A-2 and A-3 and in B-1 and B-2, and also the results of gel filtration on Sephadex G-150 (Fig. 3) show that the fractions of polysaccharides obtained were homogeneous and belonged to the glucomannan group.

The weight-average molecular weights of the glucomannans A-2 and B-1 calculated from a calibration curve based on dextrans (molecular weights 110,000, 80,000, and 40,000) [9], were 79,000 and 150,000, respectively. These results are close to the molecular weights found by sedimentation analysis [10] (76,900 and 140,000, respectively).

The monosaccharide ratios and molecular weights of the glucomannans of the species of plants studied differ from those of *Eremurus* polysaccharides studied previously [1, 11, 12].

According to their botanical classification, *E. lactiflorus* and *E. luteus* belong to the section Heningia [13, 14]. However, *E. lactiflorus*, which contains a glucomannan, is an exception from this classification, since Heningia species do not accumulate glucomannan [15]. Our results from the study of two species of *Eremurus* have shown that in actual fact both *E. lactiflorus* and *E. luteus*, which are rich in glucomannans, form exceptions to this classification.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40°C. IR spectra were taken on a UR-20 instrument in KBr tablets. Paper chromatography (PC) was performed on Filtrak FN-7, —11, and —16 papers (GDR) by the descending method using the following solvent systems (by volume): 1) butan-1-ol—pyridine—water (6:4:3); 2) ethyl acetate—pyridine—water (7:2:1); and 3) propan-1-ol—ethyl acetate—water (7:2:1). To indicate the spots we used aniline hydrogen phthalate (at 105-110°C, 10-15 min) [16]. The samples in the form of the acetates of the corresponding aldononitriles [17] were subjected to GLC on a Tsvet-101 instrument with a flame-ionization detector using a steel column (0.3 × 200 cm) filled with Chromaton N-AW, 0.200-0.250 mm, impregnated with 5% of Silicone XE-60 with helium as the carrier gas at the rate of 60 ml/min, the column temperature being 210°C.

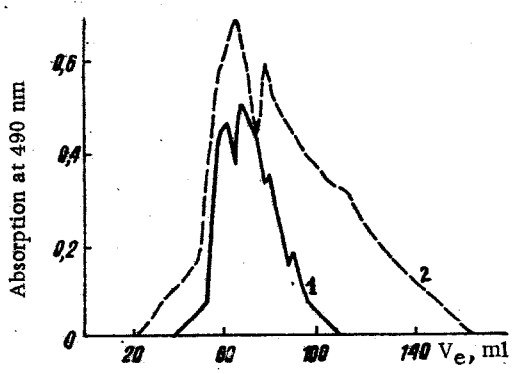

Fig. 1

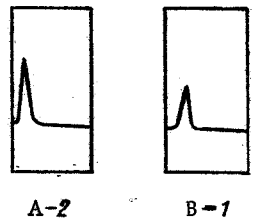

Fig. 2

Fig. 1. Gel filtration of the polysaccharides A-1 (1) and B (2) on Sephadex G-200.

Fig. 2. Sedimentograms of polysaccharides A-2 and B-1.

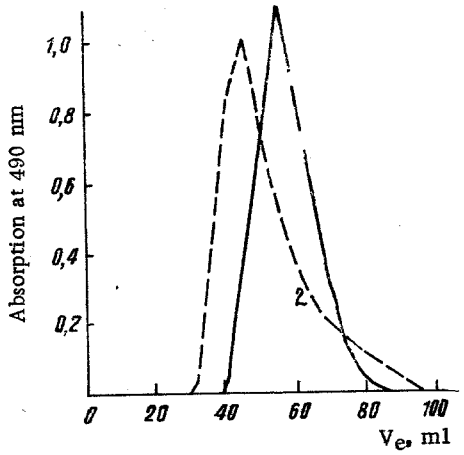

Fig. 3. Gel filtration of polysaccharides A-2 (1) and B-1 (2) on Sephadex G-150.

The polysaccharides were isolated from the comminuted (1 mm) air-dry raw material by the method of Stepanenko et al. [3] and were freed from protein substances as described by Sevag [4]. The polysaccharides were precipitated from aqueous solution with ethanol (1:3), and the precipitate was separated off by centrifugation and was dried in vacuum over P_2O_5. From 100 g of the tuberous roots of *E. lactiflorus* we isolated 13.5 g, and from 153 g of *E. luteus* 30.6 g, of polysaccharide.

Hydrolysis of the Polysaccharides. A mixture of a 50-mg sample of polysaccharide and 2.5 ml of 2 N H_2SO_4 was heated at 100°C for 14 h. The hydrolysate was neutralized with $BaCO_3$, treated with KU-2 cation-exchange resin, filtered, and dried, and the resulting syrup was subjected to PC.

Chromatography on DEAE-Cellulose. DEAE-Cellulose (polymer beads; 100 g) was treated as described by Tomoda et al. [18] and was placed in a column (36 × 3.5 cm). A solution of 2 g of the polysaccharide in 250 ml of water was deposited on the column and was eluted with water (1.5 liters). The aqueous eluate was evaporated to 200 ml and was precipitated with 600 ml of ethanol. The precipitate was centrifuged, washed with ethanol, acetone, and ether, and dried in vacuum over P_2O_5. The yield of polysaccharide A-1 was 1.52 g.

Gel Chromatography of Samples A-1 and B on Sephadex G-200. A solution of 20 mg of A-1 or B in 2 ml of water was deposited on a column (52 × 1.8 cm) filled with Sephadex G-200 and was eluted with a 0.3% solution of NaCl. The eluates were collected in 3-ml portions and were analyzed by the phenol–sulfuric acid method [19]. The results are given in Fig. 1.

Fractional Precipitation with Ethanol. With stirring, 50 ml of 96% ethanol was added to a solution of 1 g of polysaccharide A-1 in 100 ml of water. The precipitate was separated off by centrifuging and was washed with acetone and with ether and was dried. Yield 0.58 g (A-2). Similarly, 1 g of polysaccharide B gave fraction I with a yield of 0.17 g and fraction II (B-1) with a yield of 0.6 g.

Ultracentrifugation of A-2 and B-1. Aqueous solutions of the glucomannans (0.8%) were subjected to ultracentrifugation on a MOM-3170 instrument at 50,000 rpm, temperature 20°C, rate of exposure 5 min. Found: for A-2, $S = 7.69 \cdot 10^{-13}$, $D = 9.73 \cdot 10^{-7}$, mol. wt. 76,900; for B-1, $S = 9.50 \cdot 10^{-13}$, $D = 6.59 \cdot 10^{-7}$, mol. wt. 140,000.

Gel Chromatography of Glucomannans A-2 and B-1 on Sephadex G-150. Solutions of 15 mg of A-2 or B-1 in 1.5 ml of water were chromatographed with elution as described above. For A-2, $V_e = 55.6$, and for B-1, $V_e = 45.6$. The column (74 × 1.2 cm) was calibrated with dextrans having molecular weights of 110,000, 80,000, and 40,000. The free volume of the column, V_o, was 40 ml, V_e 110,000 = 47.5 ml, V_e 80,000 = 50 ml, V_e 40,000 = 53.7 ml. The molecular weights of the glucomannans were determined from a calibration curve expressing the dependence of the elution volume V_e on log M_n. The molecular weight of glucomannan A-2 was 76,000 and that of B-1 150,000.

Treatment of the PSs with Fehling's Reagent. Glucomannans A-2 and B-1 (100 mg each) were each dissolved in 10 ml of water and Fehling's reagent was added with constant stirring. The precipitates formed were washed first with 80% and then with 8% acetic acid, with water to neutrality, with acetone, and with ether. The yield of A-3 was 90 mg and of B-2 85 mg.

Hydrolysis of A-3 and of B-2. A solution of 50 mg of a PS in 2.5 ml of 72% H_2SO_4 was diluted with water to 2 N H_2SO_4 and was then heated at 100°C for 19 h. The hydrolysates were treated as described above and were subjected to PC and GLC analyses.

The determination of the O-acetyl groups in weighed samples of glucomannans A-2 and B-1 (53 mg each) that had been dried to constant weight was performed by the method of Obolenskaya et al. [5]. They amounted to 2.05% and 7.4%, respectively.

SUMMARY

Polysaccharides have been isolated from two species of *Eremurus* — *E. lactiflorus* O. Fed. and *E. luteus* Bak. — and their purification has yielded homogeneous glucomannans. They have been characterized by their molecular compositions, molecular weights, and IR spectra.

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