SUMMARY

1. It has been established that the absorption spectrum in the UV region of an aqueous solution of the LPS from *E. coli* 675 has an absorption maximum at λ_{max} 259 nm.

2. The absorption maximum of the LPS from *E. coli* 675 (259 nm) coincides with the absorption maxima (259 nm) of a number of LPS from other Gram-negative bacteria, which can be used for detecting LPSs of bacterial origin in various media.

3. It has been shown that, within the range of concentrations of 0.002-0.006%, aqueous solutions of LPSs obey the Bouguer–Lambert–Beer law, on the basis of which a method has been developed for the quantitative spectrophotometric determination of the LPS from *E. coli* 675.

LITERATURE CITED

1. J. Janda and E. Work, FEBS Letters, 16, 340 (1971).
2. N. A. Karamian, J. Pharm. Sci., 66, 723 (1977).

ACCUMULATION OF POLYSACCHARIDES UNDER THE INFLUENCE OF CHLOROCHOLINE CHLORIDE IN *Aronia melanocarpa*

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Water-soluble polysaccharides (WSPSs) have been isolated from the fruit and leaves of *Aronia melanocarpa* Elliot (black chokeberry) plants treated with chlorocholine chloride in various concentrations. It has been established that they are composed of galacturonic acid and the neutral monosaccharides galactose, glucose, arabinose, xylose, and rhamnose. In addition, another two unidentified spots of monosaccharides or their derivatives were found in the polysaccharides of the fruit, and one in those of the leaves. The ratios of the neutral monosaccharides in the WSPSs have been determined. Accumulation of the polysaccharides under the influence of various concentrations of chlorocholine chloride has been studied. It has been established that the retardant used (chlorocholine chloride) increases the accumulation of polysaccharides in the fruit by a factor of 1.5-2, but no appreciable accumulation of them was observed in the leaves.

At the present time, for regulating processes of growth and development and of the biosynthesis of biologically active substances (BASs) in plants wide use is made of synthetic bioregulators. Among them a special place is occupied by retardants [1, 2] which can exert a definite influence on the biosynthesis of BASs. There is no information in the literature on the influence of retardants — in particular, chlorocholine chloride (TUR) — on the accumulation of BASs, including polysaccharides, in *Aronia melanocarpa* Elliot (black chokeberry).

We have established previously [3] that a considerable amount of polysaccharides accumulates in the green fruit (8.52%) and in the leaves of the chokeberry in the green-fruit and fruit-fall phases (7.51 and 7.94%, respectively). The amount of polysaccharides in the ripe fruit falls to 2.37%, but by using trace elements the yield can be increased to 5% [4].

We have studied the accumulation and composition of the water-soluble polysaccharides (WSPSs) under the influence of various concentrations of chlorocholine chloride in the fruit and leaves of the black chokeberry.

The plants were treated in 1977 and 1978 in the morning hours a) with a 0.3%, and b) with a 0.6% solution, and c) twice with a 0.3% solution (0.3% × 2) of chlorocholine chloride as active substance. The first treatment was carried out at the beginning of the period of intensive growth of the shoots, and the second and third at intervals of 12-15 days after

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flowering. The consumption of the solution of the preparation was 500-1000 ml per plant. The raw material for investigation was collected during the second year of the experiment in the ripe-fruit phase.

The WSPSs were extracted with hot water. The samples of polysaccharides obtained from the ripe fruit consisted of a cherry-red powder, and those from the leaves were white with a creamy tinge. Below we give results on the influence of chlorocholine chloride on the accumulation of WSPSs in the fruit and leaves of *A. melanocarpa* (1978). As we see, the poly-

Preparation and its concentration, %	Yield of WSPSs, %	Amount of uronic anhydride, %	Ash content, %
Fruit			
Control	2,06	83,30	5,34
TUR–0,3	4,13	87,55	6,89
TUR–0,6	3,03	86,95	7,53
TUR–0,3×2	3,70	87,03	6,32
Leaves			
Control	4,29	85,91	17,31
TUR–0,3	4,53	93,23	18,70
TUR–0,6	4,86	83,83	19,87
TUR–0,3×2	4,24	90,86	15,82

saccharides were distributed nonuniformly in the fruit and leaves; the leaves contained more of them. In various concentrations, chlorocholine chloride increased the accumulation of WSPSs in the fruit by a factor of 1.5-2. In the leaves no appreciable accumulation of polysaccharides under the influence of this retardant was observed, and in one variant of the experiment (0.3% × 2) their amount actually fell somewhat as compared with the control.

The polysaccharides from the fruit of plants treated with the preparation TUR contained a considerably smaller amount of ash than the WSPSs from the leaves, which can apparently be explained by a smaller amount of mineral elements (K, Na, Ca, etc.) in them. No appreciable differences were found in the amounts of uronic anhydride in the WSPSs of the fruit, but the polysaccharides of the leaves were distinguished by some change in their amount.

The undemineralized polysaccharides were hydrolyzed with 1 N sulfuric acid. The hydrolysis products were chromatographed twice. It was established that in all the variants of the experiment the WSPSs consisted of galactose, glucose, arabinose, xylose, and rhamnose (which is in harmony with the results of previous work [3, 4]), having the respective R_f values of 0.34-0.36, 0.40-0.43, 0.46-0.49, 0.53-0.56, and 0.67-0.69. In addition to this, two unidentified spots of monosaccharides or their derivatives with R_f 0.74-0.77 and 0.91-0.94 were found in the material from the fruit, and one spot with R_f 0.61-0.63 in that from the leaves.

The ratio of neutral sugars in the WSPSs obtained under these experimental conditions were determined by the direct densitometry of the chromatograms on a Joyce Loebl type 111CS integrating microdensitometer (United Kingdom). The results (Fig. 1) were treated by the method of the statistics of variation [6].

The experimental results presented in Fig. 1 show that the predominating components in the polysaccharides of the fruit are arabinose and galactose, while in the leaves galactose predominates. Under the various conditions of the experiment, the chlorocholine chloride changed the ratios of the monosaccharides of the WSPSs found in the fruit and leaves differently. Thus, in the fruit this growth stimulator caused a marked increase (by a factor of 2.1-2.3) of the amount of glucose and a weaker but significant decrease in the amounts of galactose, xylose, and rhamnose. The relative amount of arabinose in the polysaccharides did not change. In the leaves the, as it were, opposite changes took place in the ratio of the monosaccharides determined after the addition of TUR — the amount of rhamnose increased somewhat ($P < 0.001$) and the relative amount of xylose decreased considerably (by a factor of 2-2.4). The ratios of the other monosaccharides remained at the same level as the control.

EXPERIMENTAL

The polysaccharides were extracted from the air-dry raw material (moisture content of the fruit 11-12% and of the leaves 9-10%) and were then dried in vacuum for 12 h. The ash content was determined by the combustion of samples of the polysaccharides in a muffle fur-

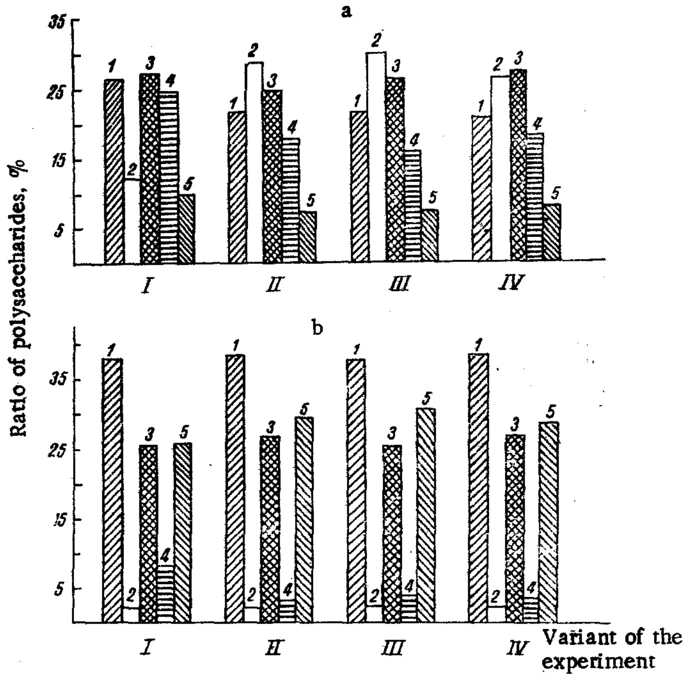

Fig. 1. Change in the ratios of the monosaccharides in the WSPSs of the fruit (a) and the leaves (b) of the black chokeberry under the influence of various concentrations of chlorocholine chloride: I) control; II) TUR, 0.3%; III) TUR, 0.6%; IV) TUR, 0.3% × 2; 1-5) galactose, glucose, arabinose, xylose, and rhamnose, respectively.

nace at 600°C. The amount of uronic anhydride was determined by complexometric titration [5].

Isolation of the Polysaccharides. The comminuted fruit and leaves of black chokeberry were extracted with hot water at 90-95°C (1:20) for 1.5 h. The aqueous extract was filtered, evaporated in vacuum to 60-80 ml, and treated with 96% ethanol (1.5 volumes). The precipitate of polysaccharides was separated off and was washed with ethanol and acetone.

Preliminary Investigation of the Products of Acid Hydrolysis of the Polysaccharides. The undemineralized polysaccharide (1 g) was dissolved in 50 ml of 1 N sulfuric acid and the solution was heated on the water bath for 9 h. The resulting hydrolysate was neutralized with barium carbonate, filtered, and evaporated in vacuum. The hydrolysis products of the WSPSs were investigated by descending paper chromatography in the butan-1-ol—pyridine—water (6:4:3) system for 45-50 h at 19-21°C (Leningrad type "M" ["slow"] paper, density 80 g/m^2). The monosaccharides were revealed with aniline phthalate with heating at 105-110°C for 10 min.

SUMMARY

1. Water-soluble polysaccharides belonging to the class of pectin substances and having different monosaccharide ratios have been isolated from the ripe fruit and leaves of the black chokeberry.* The predominating monosaccharides of the polysaccharides of the fruit were arabinose and galactose, while galactose predominated in those of the leaves.

2. Chlorocholine chloride increased the yield of polysaccharide from the fruit by a factor of 1.5-2, increased the ash content of all the WSPSs isolated from the fruit and leaves, and changed the ratio of monosaccharides in them differently. A chlorocholine chloride concentration of 0.3% acted most effectively on the accumulation of polysaccharides.

LITERATURE CITED

1. A. V. Kaplya, T. A. Moroz, and A. I. Ternavskii, The Physiology of the Action of Retardants on Fruit Crops [in Russian], Kiev (1978), p. 150.

*A. I. Yakovlev and D. D. Peskov assisted us in the isolation of the polysaccharides and in the densitometry of the chromatograms.

2. V. P. Deeva and Z. I.Sheler, The Physiology of the Resistance of Varieties of Plants to Herbicides and Retardants [in Russian], Minsk (1976), p. 245.
3. A. I. Yakovlev and E. G.Martynov, Khim. Prir. Soedin., 85 (1979).
4. A. I.Yakovlev and E. G. Martynov, Khim. Prir. Soedin., 857 (1977).
5. Z. K. Karakeeva, R. Sh. Abaeva, and G. B. Aimukhamedova, Izv. Akad. Nauk KirgSSR, No. 1, 57 (1976).
6. P. F. Rokitskii, Biological Statistics [in Russian], Minsk (1973), p. 320.

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 $\eta_{rel} = 20.5$ $[\alpha]_D^{20}$ −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 $\eta_{rel} = 9.66$. 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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