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INFLUENCE OF TRACE ELEMENTS ON THE ACCUMULATION OF ANTHOCYANINS IN THE FRUIT OF *Aronia melanocarpe*

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It has been established that in addition to vitamins, catechins, and flavonoids, the active substances of the fruit of *Aronia melanocarpa* Elliot. (black chokeberry) are anthocyanins — substances possessing a high biological activity [1].

The aim of our work was to study the accumulation of anthocyanins in the ripe fruit of the black chokeberry under the influence of trace elements and to investigate their qualitative composition.

Foliar feeding was carried out with 0.03% solutions of: H_3BO_3; $ZnSO_4 \cdot 7H_2O$; $CoSO_4 \cdot 7H_2O$; $CuSO_4 \cdot 5H_2O$; $(NH_4)_2MoO_4$; $FeSO_4 \cdot 7H_2O$; and $MnSO_4 \cdot nH_2O$ by a method described previously [2] in the "Kiritsy" sovkhoz [communal farm] in the Spassk region of the Ryazan oblast and at the young naturalists's mountain station.

The anthocyanin substances were isolated from the crude ripe fruit with 1% HCl in methanol (1:99) [3]. The extract was evaporated in vacuum and was studied by partition paper chromatography (Leningrad type "M" ["slow"] paper) in the n-butanol—acetic acid—water (4:1:5) system. In all variants of the experiment three anthocyanin substances were detected, having R_f 0.35, 0.44, and 0.67 and with the respective UV spectra: $\lambda_{\max}^{CH_3OH + 1\% HCl}$ 531, 535, and 537 nm [4]. After the hydrolysis of these substances with 2 N HCl for 30 min [5] and rechromatography of the hydrolysis products isolated by isoamyl alcohol, the three anthocyanin substances formed one and the same aglycone, which was identified as cyanidin, having R_f 0.62. UV spectra: $\lambda_{\max}^{C_2H_5OH}$ 545 nm and $\lambda_{\max}^{CH_3OH + 1\% HCl}$ 542 nm [3, 4].

The amounts of the anthocyanins in the ripe fruit of the 1973-1976 harvests were determined as cyanidin by the photocolorimetric method [6]. The results of the analysis (in % of the dry matter) were treated by the statistics of variations [7].

It was established that Co, Zn, B, Mn, Mo, Cu, and B + Zn increase the accumulation of anthocyanins by 7, 9, 11, 12, 13, 19, and 20%, respectively.

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PROANTHOCYANIDINS OF THE LEAVES OF *Laurocerasus officinalis*

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We have previously reported the isolation from the leaves and flowers of *Laurocerasus officinalis* Roem. (*Prunus laurocerasus*; common laurel cherry) of catechins and anthocyanidins and their identification [1, 2]. Continuing our investigation of the leaves of this plant, we have isolated two more compounds (I, II) by column chromatography on cellulose. Elution was performed with distilled water [3].

The compounds obtained gave the qualitative reactions characteristic for leucoanthocyanidins with the vanillin reagent and with p-toluenesulfonic acid, and on being heated with the leucoanthocyanidin reagent they were converted into the corresponding anthocyanidins [4, 5].

In the butan-1-ol—acetic acid—water (40:12:28) solvent system, compounds (I) and (II) ha R_f 0.55 and 0.50, and in 2% acetic acid 0.44 and 0.46, respectively. The UV spectra [λ_{max} (ethanol), 280 nm] and IR spectra [3400-3000 cm^{-1} (OH), 1615-1600 cm^{-1} (C_6H_5), and 1000 cm^{-1} (C—C)] of the two compounds were identical.

The anthocyanidins obtained from the compounds (I) and (II) by heating with the leucoanthocyanidin reagent were studied chromatographically and spectrally. They had R_f 0.58 in the acetic acid—hydrochloric acid—water (30:35:10) solvent system and 0.32 in the same system at a ratio of 5:1:5. The absorption maxima in the visible region of the spectrum were also identical: λ_{max} (ethanol) 545 nm, (+ $AlCl_3$) 562 nm. These results agree with the chromatographic and spectral characteristics of authentic cyanidin.

After acid hydrolysis [6] of the initial compounds, (+)-catechin for compound (I) and (+)-catechin and (—)-epicatechin for compound (II) were found in the hydrolysates by paper chromatography with markers.

In the study of the products of alkaline cleavage [7] of compounds (I) and (II), phloroglucinol and protocatechuic acid were detected, being identified by comparison with authenti samples on paper chromatography.

On the basis of the results obtained it has been established that compound (I) is a dimer consisting of (+)-catechin and leucocyanidin and (II) is a trimer the monomers of whic are (+)-catechin, (—)-epicatechin, and leucoanthocyanidin. The ease of liberating the monomers on the acid hydrolysis of (I) and (II) with dilute acid and literature information show that the monomers are linked through a C_4—C_8 bond [7-9].

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