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CHROMATOGRAPHIC INVESTIGATION OF THE ANTHOCYANIN PIGMENTS OF THE FRUIT OF SOME SPECIES OF BARBERRY

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The fruits of various species of barberry Berberis L. are used in medicine, the food industry, and Eastern cookery [1, 2]. Among the most important biologically active compounds are the anthocyanins which have so far been studied inadequately.

The fresh fruits of five species of barberry (Berberis vlugaris L., B. sieboldii Miq., B. sphaerocarpa Kar., B. integerrima Bunge., and B. coreana Palib.), gathered in the Central Botanical Garden of the Academy of Sciences of the Belorussian SSR in the phase of complete ripeness (20-100 g) were freed from seeds and were exhaustively extracted by steeping in a 1% solution of HCl in 70% ethanol at +4-5°C. The extracts obtained were chromatographed by the ascending method on FN 7 paper (GDR) using two solvent systems: 1) butan-1-ol-CH_3COOH (glac.)–water (7:2:5) (upper phase), and 2) CH_3COOH (glac.)–HCl (conc.)–water (3:1:8) [3]. The spots of the anthocyanin pigments were cut out from the paper and were eluted three times in the dark with a 0.1% solution of HCl in ethanol for 30 h.

The anthocyanin substances, which were deposited on the paper in the form of a band, were purified by rechromatography in the second solvent system. The purified anthocyanin glycosides were subjected to spectrophotometry and to acid hydrolysis (2 N HCl; 30 min) in order to obtain the anthocyanidins and the sugar residues. The aglycones and the sugars were identified by paper chromatography with authentic samples in the following solvent systems; 3) Forestal's system, and 4) butan-1-ol–pyridine–water (6:4:3) [4].

It was shown that the anthocyanins of the fruits of all the species of barberry studied were based on five aglycones – cyanidin, pelargonidin, petunidin, peonidin, and delphidinin – comined with the sugars glucose and rutinose, which we identified by paper chromatography with authentic samples and by spectral analysis [4].

Using qualitative reactions with aluminum chloride and with lead acetate and the spectral characteristics of the substances [4], and also by a paper chromatography with authentic samples of anthocyanin glycosides, it was shown that the barberry fruits contained the following glycosides: cyanidin 3-glucoside, pelargonidin 3-glucoside, petunidin 3-glucoside (only in B. vulgaris); and, additionally, peonidin 3-glucoside was found in the fruits of B. sieboldii and B. coreana. The fruits of B. sphaerocarpa and B. integerrima contained delphinidin 3-glucoside and cyanidin 3-rutinoside, in addition to the four glycosides mentioned above.

We are the first to have shown the presence of the last four anthocyanin glycosides in fruits of the five species of barberry.

Previously the literature had reported only the presence of cyanidin 3-glucoside in the fruit of B. vulgaris [5] and, of pelargonidin 3-glucoside, as well, in B. thunbergi [6].

On comparing the properties of the glycosides that we had isolated we also used authentic samples of anthocyanin glycosides that we isolated by preparative chromatography from the fruits of the blackcurrant, the pine strawberry, and the large cranberry.

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ANTHOCYANIN GLYCOSIDES OF THE FRUITS OF THE CULTIVATED BOG BILBERRY

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In connection with the fall in the commercial stocks of the fruit of the wild bog bilberry Vaccinium uliginosum L. In the European part of the USSR, work on the plantation cultivation of high-quality characterized by a considerable productivity, a stable yield, and large size of the fruit rich in biologically active compounds is acquiring enormous importance [1]. An improved variety of bilberry has been isolated in the USA and Canada by the hybridization of, mainly, two tall species – V. corymbosum L. and V. australe Small. [2, 3].

The anthocyanin pigments of the fruits of the Rancocas and Gerbert [Herbert (?)] varieties grown at the trial and experimental base of the central botanical garden of the Academy of Sciences of the Belorussian SSR (mountain settlement of Gantsevichi, Brest province) have been investigated.

The dry comminuted fruit (10-15 g) was extracted with a 1% solution of HCl in 70% ethanol by steeping in the refrigerator at +4-5° for 30 h.

To find the qualitative composition of the anthocyanin aglycones of the bog bilberry, the extract of the fruit was subjected to acid hydrolysis. It was established that the aglycones of the anthocyanin glycoside consisted of delphinidin, petunidin, malvidin, cyanidin, and peonidin, which were identified by comparison with authentic samples using paper chromatography and also on the basis of the results of spectral analysis in the visible part of the spectrum [4].

The anthocyanin extracts obtained were subjected to ascending two-dimensional chromatography on Filtrak FN 7 paper (GDR) in the solvent systems: 1) butan-1-ol–CH_3COOH(glac.)–water (3:1:1) and 2) CH_3COOH(glac.)–HCl (conc.)–water (3:1:8).

It was established that the fruits of both varieties of bog billberry contained 16 anthocyanin glycosides, i.e., their qualitative compositions were identical eight glycosides (present in the fruit in the largest amount by visual estimation) were studied in detail. The spots of the glycosides on the chromatograms were cut out, and were repeatedly extracted with 90% ethanol containing 0.1% of HCl (conc.), and purified in system 2. The purifird anthocyanin glycosides were identified from the results of a study of their absorption spectra in 96% ethanol containing 0.01% HCl (conc.) and also after the acid hydrolysis followed by extraction with isoamyl alcohol of their aglycones, which were identified by paper chromatography with authentic samples in solvent system 3) CH_3COOH (glac.)–HCl(conc.)–water (30:3:10), while the sugar residues were identified in system 4) butan-1-ol–pyridine–water (6:4:3) [4].

It was shown that the anthocyanins of the fruits of the cultivated bog bilberry were represented by four aglycones – delphidinin, cyanidin, petunidin, and malvidin – combined with sugar residues of D-galactose, L-arabinose, and D-glucose [4].

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