Chemistry of Natural Compounds, Vol. 41, No. 2, 2005

ANTHOCYANS FROM FRUIT OF SOME PLANTS OF THE CAPRIFOLIACEAE FAMILY

V. I. Deineka, V. N. Sorokopudov, L. A. Deineka, E. I. Shaposhnik, and S. V. Kol′tsov

UDC 543.54:547.973

*The qualitative and quantitative compositions of anthocyans from fruit of plants of the Caprifoliaceae family grown under conditions of Belgorod region were investigated. The plants included elder species [*Sambucus nigra *L. **(I)**,* S. canadensis ***(II)**, and* S. canadensis f. sceletoniana ***(III)**],* Viburnum opulus *L., and* Lonicera caerulea *L. It has been found that the principal anthocyans of **I** are cyanidin-3-sambubioside (52.5%) and cyanidin-3-glucoside (37.1%) whereas from the other two elder species the principal component was cyanidin-3-sambubioside-5-glucoside, acylated p-coumaric acid, 70.9 **(II)** and 54.3% **(III)**. Fruit of the other plants did not contain acylated anthocyans. The content of anthocyans in fresh or dried fruit was determined by spectrophotometry.*

Key words: anthocyans, HPLC, *Sambucus nigra*, *S. canadensis*, *S. canadensis f. sceletoniana*, *Viburnum opulus*, *Lonicera caerulea*, freshly collected and dried fruit, hydrolysis.

Black elder (*Sambucus nigra* L.) is a traditional plant of Belgorod region. The healing properties of the fruit and flowers of this plant have been known since long ago. It is recommended in modern medicine [1, 2].

We investigated fruit of black elder and those of *S. canadensis* L. and its serrate-leafed variety *S. canadensis f. sceletoniana*, which were introduced to the Botanical Garden of Belgorod University; cranberry, *Viburnum opulus* L., also a traditional plant of the region; and finally, blue-fruit honeysuckle, *Lonicera caerulea* L.

Table 1 gives the results. The qualitative composition of anthocyans found by us agrees with the literature [3-6]. The principal components for the black-elder anthocyan complex were unacylated 3-glycosides of cyanidine (sambubioside, Cy-3-Sam, and the glucoside, Cy-3-Glu) whereas for the other elders the acylatd derivative, Cy-3-Sam*-5-Glu, the structure of which was determined [3] as cyanidin-3-(6-*p*-coumaroyl-2-xylosylglucoside)-5-glucoside, was significantly dominant. In the first instance, a relatively small quantity of unacylated diglycosides, Cy-3-Sam-5-Glu and Cy-3,5-diGlu, was also detected. The difference in the anthocyan composition of black elder, on the one hand, and the other elders, on the other, is easily observed also from the bathochromic shift of the absorption wavelength maximum of the total pigments in aqueous HCl solutions (by 5 nm, from 505 to 510 nm).

The structures of the detected pigments agreed with the literature data according to results from an investigation of the acid-hydrolysis products of the extracts. We note that the main acylated component of the extracts already after 15-20 min practically completely loses the xylosyl unit (like unacylated Cy-3-Sam-5-Glu) whereas the unacylated hydrolysis products in all instances accumulate less than acylated Cy-3-Glu and the final product, the aglycon.

Potentiometric titration showed that fruit of *S. canadensis* contains 2-3 times less acids (0.007 mol-eq per 100 g) than black elder (0.017-0.022 mol-eq per 100 g).

The anthocyan composition of fruit from *L. caerulea* is much simpler. The principal component is cyanidine 3-glucoside; the next most plentiful component, cyanidine 3-rutinoside, Cy-3-Rut. Fruit of *V. opulus* contain cyanidine 3-glucoside and 3-rutinoside and traces of cyanidine 3-sambubioside. The next most plentiful component of the complex is a cyanidine derivative constructed from arabinose, cyanidin-3-arabinolysglucoside [5], which was absent in the glycoside radicals of fruit anthocyans of other studied plants in this family.

Belgorod State University, 308015, Belgorod, ul. Pobedy, 85. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 132-133, March-April, 2005. Original article submitted November 1, 2004.

 0009-3130/05/4102-0162

TABLE 1. Anthocyan Composition of Fruit from Plants of the Caprifoliaceae Family

Cyanidine derivatives	*Viburnum opulus*	*Lonicera caerulea*	*Sambucus*		
			canadensis f. sceletoniana	*canadensis*	*nigra*
	mol, % from peak area (±1.1-0.8%)				
3-Sam-5-Glu	0	0	32.2	14.5	8.1
3,5-diGlu	0	0	7.4	11.0	1.0
3-AraGlu	32.2	0	0	0	0
3-Sam	0.5	0	0.5	0.6	52.5
3-Glu	51.2	84.8	0	0.3	37.1
3-Rut	12.4	6.4	0	0.3	1.0
3-Sam*-5-Glu	0	0	54.3	70.9	0
Others	3.7	8.8	5.6	2.4	0.3
Total anthocyans g/100 g fruit:					
fresh	0.022-0.029	-	-	0.45-0.84	0.42-0.86
dried	-	0.75-0.95	1.40-1.80	1.7-2.0	2.20-2.80

*Designations of anthocyans are given in the text.

The absolute content of anthocyans (calculated for cyanidin-3-glucoside) in fresh fruit of black elder was exceedingly nonuniform for samples from various collection times (September-October 2004) and in various habitats (from the shade of other trees to solitary bushes). The anthocyan content in the studied fruit even with a large accumulation was noticeably less than in the 2003 season (0.9-1.2 g per 100 g fruit). This is probably due to the very unfavorable weather conditions during the last year. The anthocyan content in dried fruit from all three studied elder species remained high. This enabled drying to be used as a technical step in isolating the food dyes.

EXPERIMENTAL

Anthocyans were extracted from freshly collected fruit of elder and *V. opulus* (September-October 2004) and from dried fruit of *L. caerulea* (collected in July 2004). We used formic acid (10 vol%) in water for the extraction. The absolute anthocyan content was determined by spectrophotometry [7] by diluting the aqueous extracts with HCl solution until the pH was 1.0-1.3 (KFK-3-01 spectrophotometer). Special investigations showed that neither the position of the absorption maximum nor the extinction coefficient of the anthocyans changed for a formic-acid content (entering the solution from the extract) up to 2 vol%. Fruit was dried in air without exposure to direct sunlight.

The qualitative composition of the anthocyans was determined using HPLC [8]. Direct chromatographic analysis of the extract was impossible because of the high content of ballast substances in it that could quickly spoil the precolumn. Their content in the extract was reduced by adding acetone to the mixture (about 40 vol%). Acetone was removed from the filtrate in vacuo before chromatography. The anthocyan structures were confirmed using hydrolysis (in 3-5% HCl solution on a boiling-water bath). The hydrolysis products were separated from the HCl by sorption on DIAPAK cartridges packed with reversed-phase sorbent. The sorbent was activated by washing with acetone (1 mL) and conditioned with extractant (1 mL, 10% formic acid in water). Then hydrolysate (about 0.5 mL) was sorbed, washed with extractant (2.0 mL), and eluted by a mixture of extractant and CH_3CN (1:0.5 by vol).

Two different isocratic regimes were used for chromatographic determination of the hydrolysate components: 9-12 vol% CH_3CN and 10 vol% formic acid in water for investigating unacylated components and 18-24 vol% CH_3CN and 10 vol% formic acid in water for investigating acylated anthocyans.

REFERENCES

1. V. V. Kovalenko, *Apothecary Garden* [in Russian], Uradzhai, Minsk (1994).
2. M. D. Mashkovskii, *Medicinal Preparations* [in Russian], in 2 vols., Vol. 1, Meditsina, Moscow (1985), p. 468.
3. O. P. Johansen, W. M. Andersen, W. Nerdal, and D. W. Aksnes, *Phytochemistry*, **30**, 4137 (1991).
4. K. J. Bronnum-Hansen, *J. Chromatogr.*, **262**, 385 (1983).
5. E. N. Kananykhina and I. V. Pilipenko, *Khim. Prir. Soedin.*, 118 (2000).
6. T. Watanabe, A. Yamamoto, S. Nagai, and S. Terabe, *Anal. Sci.*, **14**, 839 (1998).
7. M. M. Giusti and R. E. Wrolstad, in: *Current Protocols in Food Analytical Chemistry*, S. King, M. Gates, and L. Scaletter, eds., John Wiley & Sons, Inc., New York (2000).
8. V. I. Deineka, A. M. Grigor′ev, V. M. Staroverov, and O. N. Borzenko, *Khim. Prir. Soedin.*, 137 (2003).