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ANTHOCYANINS IN FRUITS OF *VACCINIUM JAPONICUM*

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Key Word Index—*Vaccinium japonicum*; Ericaceae; anthocyanins; cyanidin-3-arabinoside; pelargonidin-3-arabinoside; chemotaxonomy.

Abstract—Cyanidin-3-arabinoside (54%) and pelargonidin-3-arabinoside (39%) were the main anthocyanins isolated from berries of *Vaccinium japonicum*. In addition smaller amounts of 3-galactosides of cyanidin (5%) and pelargonidin (2%) were found. The total anthocyanin content in the fruit averaged 113 mg/100 g fresh fruit. This is the first report of pelargonidin derivatives in the genus *Vaccinium*.

INTRODUCTION

From a chemotaxonomic point of view, Harborne [1] has considered the pattern of the anthocyanin contents of ericaceous plants to be very consistent; 3-galactosides and 3-arabinosides are the most widely distributed types and characterize the family. Later Ballinger *et al.* [2] suggested that the genus *Vaccinium* may be characterized by the general presence in its fruits of 3-monoglycosides in which the aglycones delphinidin, petunidin, malvidin, peonidin and cyanidin are combined with the sugars galactose, arabinose and glucose. However, their conclusion is that fruits of the genus do not appear to be uniform, since the anthocyanin content varies with subgeneric classification. This conclusion is partly based on the results of Troyan and Borukh [3], which have recently been questioned [4, 5].

Vaccinium japonicum is native to Eastern Asia. This study was designed to investigate the possibility that the scarlet colour of its edible fruits might indicate the presence of pelargonidin derivatives.

RESULTS AND DISCUSSION

The anthocyanin extract of *V. japonicum* was applied to HPLC both before and after treatment with Amberlite CG-50 resin, and the same four peaks were detected. Together with chromatographic data, the spectra suggested that the pigments corresponding to peaks 1 and 3 were based on cyanidin, while peaks 2 and 4 were pelargonidin derivatives. The spectra indicated the absence of sugar in the 5-position and of acylation with aromatic acids.

The four anthocyanins were separated by preparative HPLC, and exposed to acid hydrolysis, deacylation with alkali and H_2O_2 oxidation. They were thus identified as cyanidin-3-arabinoside (54%) pelargonidin-3-arabinoside (39%), cyanidin-3-galactoside (5%) and pelargonidin-3-galactoside (2%). The total anthocyanin content in the fruit averaged 113 mg/100 g fresh fruit.

Pelargonidin-3-arabinoside is a rare pigment, having been detected before in follicles of *Sterculia parviflora* (Sterculiaceae) [6], in flowers of *Erica regia* (Ericaceae) [7], in flowers of *Epacris impressia* and *Acrotriche serrulata* and in fruits of *Cyathodes petiolaris* (all Epacridaceae) [8]. There have been suggestions for placing *V. japonicum* either in its own genus *Hugeria* or in the subgenus *Oxycoccoides* [9, 10]. Among all *Vaccinium* species examined, the anthocyanin pattern of *Vaccinium japonicum* most closely resembles *V. macrocarpon*, which is indeed in the subgenus *Oxycoccoides* [11]. These new results indicate that the hypothesis of Ballinger *et al.* [2] needs to be reformulated as "The genus *Vaccinium* is characterized by the general presence in its fruits of some of the various 3-monoglycosidic combinations of the six common aglycones, including pelargonidin."

EXPERIMENTAL

Plant material. Berries of *V. japonicum* were collected in September 1985 from the Botanical Garden of the University of Bergen, Norway. The identity of the plant was verified by D. O. Øvstedal and voucher specimens are deposited in BG.

Authentic compounds. 3-Galactoside and 3-arabinoside of cyanidin were obtained from cowberries (*Vaccinium vites-idaea*) [4], while pelargonidin-3-galactoside was isolated from fruits of *Cornus mas* [12].

Extraction and purification. Ripe berries were macerated in a blender with MAW, MeOH–HOAc–H_2O (10:1:9). After filtration the residue was re-extracted twice with MAW. The combined anthocyanin solns were washed with petrol and EtOAc, filtered, concd under reduced pressure at 35° and treated with Amberlite CG-50 resin [13].

HPLC. HPLC was performed with a solvent delivery system (SP-8700, Spectra-Physics), and automated sampling system (ISS-100, Perkin–Elmer) and a photodiode array detector (HP-1040A, Hewlett-Packard) interfaced to a computer and a disc drive. Two solvents were used for elution: HCO_2H–H_2O, (1:9) (A) and HCO_2H–H_2O–MeOH, (1:4:5) (B). The anthocyanins were detected at 515 ± 25 nm. Repeated passage of the concd purified anthocyanin mixture through a semiprep. column, Supelcosil LC-18 (25 × 1 cm, Supelco) 5 μm, separated the anthocyanins. The elution profile was: 0–10 min, 10–50% B in A (linear gradient); 10–35 min, 50–85% B in A (linear gradient), and the flow rate was 3.5 ml/min. The elution profile of the analytical column, Supelcosil LC-18 (25 cm × 4.6 mm, Supelco) 3 μm, was 0–21 min, 10–80% B in A (linear gradient), and the flow rate was

1.5 ml/min. The total amount of anthocyanins was calculated as cyanidin-3-galactoside from a standard curve.

TLC. TLC of pigments was carried out on cellulose plastic sheets (Merck, 5565) with BAW (*n*-BuOH–HOAc–H_2O, 6:1:2), HW (conc. HCl–H_2O, 3:97), AW (HOAc–H_2O, 15:85) and FHW (HCO_2H–conc. HCl–H_2O, 6:1:5). R_f values for cyanidin-3-arabinoside, pelargonidin-3-arabinoside, cyanidin-3-galactoside and pelargonidin-3-galactoside were 0.26, 0.41, 0.14 and 0.24 in BAW; 0.04, 0.08, 0.05 and 0.09 in HW; 0.37, 0.49, 0.37 and 0.49 in AW; 0.57, 0.69, 0.56 and 0.69 in FHW.

Degradation procedures. Acid hydrolysis, deacylation with alkali and H_2O_2 oxidation were according to standard procedures [14].

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FLAVONOL GLYCOSIDES FROM *CALLITRIS GLAUCA*

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Key Word Index—*Callitris glauca*; Cupressaceae; myricetin 7-arabinoside; kaempferol 5-rhamnoside; flavonol glycosides.

Abstract—From the leaves of *Callistris glauca* myricetin 7-arabinoside, quercitrin, kaempferol 5-rhamnoside, a quercetin arabinoside, quercetin, kaempferol, galangin and shikimic acid were isolated. The natural occurrence of myricetin 7-arabinoside has not previously been reported.

INTRODUCTION

A reinvestigation of the leaves of *Callitris glauca* R.Br. has resulted in the isolation of a new glycoside, myricetin 7-*O*-arabinoside (**1**), together with the known substances quercetin 3-*O*-rhamnoside (**2**), a quercetin arabinoside (**3**), kaempferol 5-rhamnoside (**4**), quercetin, kaempferol, galangin and shikimic acid. Kaempferol 5-rhamnoside was recently reported from this plant for the first time with other flavonoid constituents [1].

RESULTS AND DISCUSSION

The aqueous layer of a methanolic leaf extract of *C. glauca* was extracted with ethyl acetate and concentrated to give yellow crystals of shikimic acid (co-TLC, mmp and spectral data). The concentrated filtrate upon repeated CC and TLC on silica gel followed by crystallization afforded four compounds (**1**–**4**). Myricetin 7-arabinoside (**1**) on acid hydrolysis gave myricetin [2, 3] and arabinose (PC). A comparative study of the ^{1}H NMR spectral data of the acetate of **1** and acetylated myricetin indicated glycosylation at C-7. The two doublets (J = 2.5 Hz) at δ6.46 and 6.75 due to C-6 and C-8 protons of acetylated **1** were shifted downfield to δ6.50 and 6.90 in the sugar-free myricetin acetate. This downfield shifting in ring A and the greater shift of the C-8 proton than that of C-6 showed that the change of electron density was in the vicinity of C-8. The mass spectrum of **1** showed no molecular ion peak but a peak of low intensity at m/z 391 (2) $[(M-60)+H]^+$ was obtained, indicating that the sugar involved in glycosylation was an aldopentose. The UV spectral data for **1** and its partially methylated aglycone in different diagnostic reagents (see