

UNUSUAL ANTHOCYANINS FROM THE EPACRIDACEAE

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Abstract—Cyanidin-3-xylosylarabinoside, cyanidin- and delphinidin-3-rhamnosylgalactoside and cyanidin-3-xylosylgalactoside have been identified during a survey of anthocyanins in the Epacridaceae. A minor delphinidin bioside, probably delphinidin-3-xylosylgalactoside, was also present.

A DETAILED survey of anthocyanins in the Epacridaceae has led to the identification of three new compounds, viz. cyanidin- and delphinidin-3-rhamnosylgalactoside in *Cyathodes glauca* (fruit) and cyanidin 3-xylosylarabinoside (cyanidin-3-epacroside) in *Cyathodes dealbata* (fruit). Two additional anthocyanins which may prove to be the rare cyanidin- and delphinidin-3-lathyrosides, were also isolated from *C. dealbata* (fruit). The presence of anthocyanins containing these unusual disaccharides is quite consistent with the widespread occurrence of 3-galactosides and 3-arabinosides in the family.

Only one of the five compounds, cyanidin-3-xylosylarabinoside, exhibited chromatographic behaviour sufficiently different from any published data to suggest that its glycosidic substituent might be unusual. Acid hydrolysis and treatment with alkaline hydrogen peroxide indicated that a disaccharide containing xylose and arabinose was attached to cyanidin at position 3. The ratio of the colour intensity of the two sugars, after spraying chromatograms with aniline hydrogen phthalate, was visually estimated as 1:1. Spectral data confirmed that no substituent was present at position 5, and spectral data and chromatographic behaviour indicated that the pigment was not acylated. A partial hydrolysis, with subsequent isolation and identification of cyanidin-3-arabinoside as the intermediate indicated the order in which the sugars were attached to the aglycone. The yield of cyanidin-3-monoside from the partial hydrolysis was small compared with that obtained from other 3-biosides isolated from the Epacridaceae, suggesting that the aglycone-sugar link in this anthocyanin is unusually labile under acid conditions.

Cyanidin-3-epacroside was detected in only two of the sixty-four species examined during the survey. It occurred as a minor pigment and its presence in each species was not consistent. The disaccharide present in this anthocyanin is of particular interest since it has not been reported previously as a substituent in any class of flavonoid. However, xyloarabinosides are known as components of polysaccharides, e.g. in corn hull and barley husk hemicelluloses, and in apple gum.¹⁻³

The identification of cyanidin- and delphinidin-3-rhamnosylgalactoside and cyanidin 3-xylosylgalactoside was carried out using standard procedures.⁴ During the gener

¹ R. MONTGOMERY, F. SMITH and H. C. SRIVASTAVA, *J. Am. Chem. Soc.* **79**, 698 (1957).

² G. O. ASPINALL and R. J. FERRIER, *J. Chem. Soc.* 4188 (1957).

³ A. J. CHARLSON, P. A. J. GORIN and A. S. PERLIN, *Can. J. Chem.* **35**, 365 (1957).

⁴ J. B. HARBORNE, *Comparative Biochemistry of Flavonoids*, Academic Press, New York (1966).

survey of anthocyanin distribution in the Epacridaceae, using 1- and 2-dimensional PC, it was not possible to predict the identity of these three compounds with any degree of confidence because of the similarity of their R_f s to those of cyanidin- and delphinidin-3-rutinoside and cyanidin-3-sambubioside, respectively. Cochromatography as a method of differentiating between the respective anthocyanins was successful only when purified pigments were chromatographed at equivalent concentrations and under identical conditions. In preparative scale chromatography it was possible to separate these pigments only by developing the chromatograms for excessively long periods. During the survey, hydrolysis of the purified pigments and analysis of the sugar components was adopted as the routine procedure.

It seems probable that the structure of the rhamnosylgalactose is analogous to rutinose (1,6- α -rhamnosyl-D-glucose), because of the similarity in R_f s of the respective parent anthocyanins. 1,6- α -rhamnosyl-D-galactose (robinobiose) has not been reported previously in anthocyanins although it is known to occur as a substituent of several flavonols (kaempferol-, quercetin- and myricetin-3-robinobioside 7-rhamnoside).⁴ From similar reasoning, it seems probable that the disaccharide present in cyanidin-3-xylosylgalactoside is structurally analogous to sambubiose (1,2- β -xylosyl-D-glucose). This disaccharide (1,2- β -xylosyl-D-galactose, lathyrose), is rare in anthocyanins and is known only from *Lathyrus odoratus* (Leguminosae),⁴ and from *Aralia* (Araliaceae).⁵ However, the supposed structures for the Epacrid disaccharides have not been confirmed because authentic references of robinobiose and lathyrose were not available for chromatographic comparison.

TABLE 1. R_f VALUES FOR ANTHOCYANINS

Compound	R_f ($\times 100$) in		
	BAW	3% HCl	WAH*
Cyanidin derivatives			
3-Xylosylarabinoside	35	21	31
3-Xylosylglucoside	30	26	41
3-Xylosylgalactoside	29	27	42
3-Rhamnosylglucoside	30	16	30
3-Rhamnosylgalactoside	28	14	28
3-Arabinoside	38	6	18
3-Galactoside	30	6	17
Delphinidin derivatives			
3-Xylosylgalactoside	22	23	40
3-Rhamnosylglucoside	21	10	21
3-Rhamnosylgalactoside	17	7	19
3-Galactoside	18	3	10

* WAH = H₂O-HOAc-conc. HCl (82:15:3).

A minor delphinidin-3-bioside was also isolated, but because of insufficient purified material, it was not possible to determine the sugars unambiguously. The R_f of the intermediate product obtained during partial hydrolysis was similar to that of delphinidin-3-galactoside, but complete hydrolysis of the contaminated pigment yielded glucose, as well as xylose and galactose. We have tentatively identified this anthocyanin as delphinidin-3-xylosylgalactoside on the basis of the hydrolysis results and also because of its co-occurrence with cyanidin-3-xylosylgalactoside in *Cyathodes dealbata*, and the parallel chromatographic behaviour of these two pigments.

⁵ K. KAWANO and S. SAKAMURA, *Agric. Biol. Chem. Japan* 36, 27 (1972).

R_f s of the five Epacrid anthocyanins reported here are shown in Table 1. The distribution of these anthocyanins, along with other anthocyanins present in the family, will be reported separately.

EXPERIMENTAL

Fresh plant material was used for all extractions and voucher specimens of the species used were placed in the Tasmanian University Herbarium.

Extraction and purification. The fresh plant material was extracted with MeOH containing a minimum quantity of HCl. The filtered extract was concentrated to a small vol. in a rotary evaporator, H₂O was added and evaporation continued until all traces of MeOH were removed. The remaining aq. solution was further diluted with a large amount of H₂O (at least 5 × the vol. of the original MeOH extract), filtered to remove H₂O insoluble materials and then passed through a column containing a weak carboxylic acid ion-exchange resin, Bio-Rex 70H⁺ (Calbiochem). The anthocyanin mixture was then eluted from the column with MAW (MeOH-HOAc-H₂O, 17:1:2) and concentrated to dryness by rotary evaporation. This preliminary treatment, while causing no apparent breakdown of the pigments, provided a mixture of anthocyanins free from bulky, viscous contaminants, and greatly facilitated the subsequent fractionation procedures. Purification of the individual anthocyanins was achieved by preparative scale paper chromatography on Whatman 3MM paper, using BAW (*n*-BuOH-HOAc-H₂O, 4:1:5, organic layer) and 5% HOAc, in succession. Some difficulty was encountered initially in the purification of delphinidin-3-rhamnosylgalactoside and cyanidin-3-xylosylarabinoside. The former was contaminated with a colourless compound which interfered with the acid hydrolysis resulting in the rapid disappearance of the aglycone within 12 min of heating, and subsequent analysis of the sugars was difficult to interpret. Cyanidin-3-xylosylarabinoside was contaminated with a yellow flavonoid. Neither contaminant could be separated adequately by PC using the normal anthocyanin solvents. However, in both cases it was possible to remove the contaminant by passing a weakly acidic, aq. solution of the partially purified anthocyanin through a short column (3 cm long × 3 cm dia.) of polyamide-celite. The anthocyanin was collected directly onto a column of the ion-exchange resin, Zeo-Karb 226H⁺ (Permutit Co.), from where it could be eluted with MAW as a pure anthocyanin. PC monitoring of the process showed that this treatment caused no alteration of the anthocyanin structure.

Identification. Identification of the anthocyanins was made using the procedures described by Harborne.⁴ The alkaline H₂O₂ oxidations were carried out according to the method of Chandler and Harper,⁶ but treatment with H₂O₂ was extended to 24 hr. The reference anthocyanins, cyanidin and delphinidin 3-rutinoside were isolated from *Trochocarpa gunnii* (Epacridaceae) and cyanidin 3-sambubioside, from *Sambucus nigra*. R_f s of the anthocyanins were determined as described previously,⁷ but using 50 µl of solution with an optical density of 1.0. Chromatograms were run in duplicate.

⁶ B. V. CHANDLER and K. A. HARPER, *Austral. J. Chem.* **15**, 114 (1962).

⁷ S. J. JARMAN and R. K. CROWDEN, *Phytochem.* **10**, 2235 (1971).