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CYANIDIN 3-ARABINOSIDE-5-GLUCOSIDE, AN ANTHOCYANIN WITH A NEW GLYCOSIDIC PATTERN, FROM FLOWERS OF "RED WING" AZALEAS

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Abstract—Cyanidin 3-arabinoside-5-glucoside, the first representative of this glycosidic class, was isolated from flowers of the azalea (*Rhododendron*) cultivar "Red Wing". Other anthocyanins present in these tissues were cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-arabinoside, and cyanidin 3,5-diglucoside.

INTRODUCTION

THE occurrence of arabinose as the sugar moiety of anthocyanins is rare.[1] Cyanidin 3-arabinoside, the only arabinoside of cyanidin that has been reported, was isolated from flowers of rhododendron (*Rhododendron* spp.[2]), cotyledons of cacao (*Theobroma cacao*[3]), and husks of barley (*Hordeum vulgare*).[4] This paper describes the isolation and characterization of cyanidin 3-arabinoside-5-glucoside, an anthocyanin with a new glycosidic pattern, obtained from the flowers of Red Wing azaleas.

RESULTS

Purification and Characterization

The anthocyanins isolated from flowers of Red Wing azaleas resolved into two bands when passed through a column of powdered nylon.[5] When these individual bands were chromatographed with BAW (butan-1-ol–acetic acid–water, 6:1:2 v/v) for 18 hr, band 1 remained homogeneous but band 2 resolved into two components designated as bands 2.1 and 2.2. The properties of the isolated anthocyanins are shown in Table 1 and Fig. 1.

Unlike bands 2.1 and 2.2, the absorption spectra of band 1 did not have the distinct shoulder to the main absorption peak in the 410–450 nm region. The absence of this shoulder is characteristic of an anthocyanin with sugar residues on the 5 hydroxyl group.[6] The shoulder was apparent for bands 2.1 and 2.2, a factor which indicated an anthocyanin with a free 5 hydroxyl group. Harborne[7] also has shown that the short wavelength peak (at about 265–280 nm) of 3,5-diglycosides is consistently less intense than that of 3-glycosides.

From the data in Table 1 and Fig. 1 it appears that band 1 is a 3,5-diglycoside of cyanidin and that bands 2.1 and 2.2 are 3-glycosides of cyanidin.

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[4] M. METCHE and E. URION, *Compt. Rend.* **252**, 356 (1961).
[5] M. K. SEIKEL, *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 34. Pergamon Press, Oxford (1962).
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[7] J. B. HARBORNE, *Phytochem.* **2**, 85 (1963).

TABLE 1. PROPERTIES OF ANTHOCYANINS ISOLATED FROM RED WING AZALEAS

Compound	Color in u.v. light	λ_{max} MeOH—HCl (nm) Band 1	Band 2	$AlCl_3$ shift $\Delta\lambda$ (nm)	Ratio (%) $\frac{E_{440 nm}}{E_{max}}$	BAW*	BH*	WHF*	AH*	EFW†
Azalea anthocyanins										
Band 1	Red	278	525	45	14	0·22	0·10	0·49	0·48	0 22
Band 2.1	Purple	282	526	44	22	0·35	0·30	0·19	0·32	0·60
Band 2.2	Purple	282	526	44	22	0·33	0·23	0·22	0·32	0·53
Authentic anthocyanins										
Cyanidin 3,5-di-glucoside	Red	278	525	45	13	0·21	0·08	0·45	0 44	0·19
Cyanidin 3-glucoside	Purple	282	526	44	22	0·34	0·25	0·21	0·32	0·56
Cyanidin 3-rutinoside	Purple	282	526	44	23	0·35	0·26	0·41	0 50	0·39

* Determined by thin-layer chromatography on cellulose (MN-300) plates 250 mμ thick. BAW, butan-1-ol–acetic acid–water (6:1:2 v/v); BH, butan-1-ol–2 N HCl (1:1 v/v); WHF, water–HCl–formic acid (8:4:1 v/v); AH, acetone–2 N HCl (1:3 v/v).

† Determined by thin-layer chromatography on silica gel-H-plates 250 mμ thick: in ethyl acetate-formic acid–water (70:15:15 v/v).

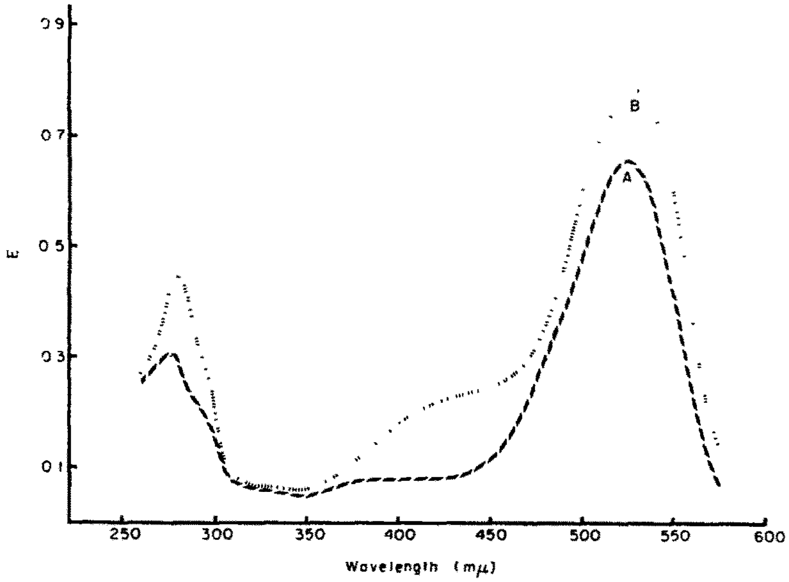

FIG. 1. ABSORPTION SPECTRA OF ANTHOCYANINS IN METHANOLIC 0·01% (w/v) HCl ISOLATED FROM FLOWERS OF RED WING AZALEAS.

A, band 1; B, band 2.1 or 2.2.

Acid Hydrolysis

Both bands 2.1 and 2.2 yielded only cyanidin (Table 2). The sugar moiety from band 2.1 was arabinose, and that from band 2.2 glucose and galactose in a molar ratio of approximately 1 to 1.

TABLE 2. PROPERTIES OF THE ANTHOCYANIDIN OBTAINED FROM THE ACID HYDROLYSIS OF THE ANTHOCYANINS ISOLATED FROM RED WING AZALEAS

Compounds	λ_{max} in MeOH—HCl (nm)	AlCl shift $\Delta\lambda$ (nm)	R_f values in*		
			AHW	FHW	BH
Azalea anthocyanidin					
Band 1	535	45	0·62	0·38	0·66
Band 2.1	535	45	0·62	0·38	0·66
Band 2.2	535	45	0·62	0·38	0·66
Authentic anthocyanidins					
Cyanidin	535	45	0·60	0·39	0·65
Delphinidin	546	34	0·42	0·23	—
Malvidin	542	0	0·72	0·44	—
Pelargonidin	520	0	0·77	0·50	—
Peonidin	532	0	0·77	0·46	—
Petunidin	543	39	0·58	0·34	0·49

* On Whatman No. 1 paper; AHW, acetic acid–HCl–water (30:3:10 v/v); FHW, formic acid–HCl–water (5:2:3 v/v); BH, butan-1-ol–2 N HCl (1:1 v/v).

Cyanidin 3-glucoside and cyanidin 3-galactoside are difficult to separate chromatographically but they can be resolved by prolonged development with butan-1-ol–acetic acid–water.[8] When band 2.2 was developed on Whatman No. 1 paper with this solvent for 48 hr, it resolved into two distinct spots. Band 2.2 is therefore a mixture of cyanidin 3-glucoside and cyanidin 3-galactoside, while band 2.1 is cyanidin 3-arabinoside. Cyanidin 3-arabinoside and cyanidin 3-galactoside have been previously reported in flowers of *Rhododendron* species.[2]

TABLE 3. SUGARS FROM ACID HYDROLYSIS OF ANTHOCYANINS ISOLATED FROM RED WING AZALEAS

Compounds	R_g values in*			Color with aniline hydrogen phthalate
	EPW	BEW	Phenol	
Sugars from anthocyanins:				
Band 1	1·02	0·99	1·00	Brown
	1·52	1·15	1·42	Red
Band 2.1	1·50	1·16	1·42	Red
Band 2.2	0·84	0·90	1·08	Brown
	1·00	1·00	1·00	Brown
Authentic sugars				
Galactose	0·84	0·92	1·08	Brown
Glucose	1·00	1·00	1·00	Brown
Arabinose	1·50	1·16	1·42	Red
Lyxose	2·13	1·35	1·34	Red
Xylose	1·93	1·30	1·24	Red
Rhamnose	2·84	1·72	1·62	Brown

* On Whatman No. 1 paper; EPW, ethyl acetate–pyridine–water (8:2:1 v/v); BEW, butan-1-ol–ethanol–water (40:11:19 v/v); phenol, phenol–water (73:27 w/w).

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[8] R. ROBINSON and H. SMITH, *Nature* **175**, 634 (1955).

Band 1 was crystallized according to the procedure of Shibata and Sakai[9] and after acid hydrolysis yielded only cyanidin (Table 2). Sugars obtained were glucose and arabinose (Table 3).

Hydrogen Peroxide Oxidation

Karrer and de Meuron,[10] and Chandler and Harper,[11] showed that controlled peroxide oxidation of anthocyanins permitted the specific identification of sugar groups at the C-3-position. Providing the pH of the solution was kept below 8·5, the reaction was very clean, no hydrolysis of oligosaccharides occurred, and sugar groups at positions other than C-3 were not detached. The peroxide oxidation of band 1 yielded glucose and arabinose and not an intact oligosaccaride. This suggested that band 1 contained more than one component. When band 1 was developed on Whatman No. 1 paper with BAW for 72 hr, it resolved into two distinct spots designated as 1.1 and 1.2. A sufficient amount of each of the components of band 1 was obtained by preparative paper chromatography. The absorption spectra of bands 1.1 and 1.2 were identical to that of band 1 (Fig. 1). Acid hydrolysis of band 1.1 yielded cyanidin, arabinose, and glucose in a molar ratio of 1:1·2:1·2. The peroxide oxidation yielded only arabinose. Band 1.1, therefore, is cyanidin 3-arabinoside-5-glucoside. Acid hydrolysis of band 1.2 yielded cyanidin, and glucose in a molar ratio of 1:2·2. The peroxide oxidation yielded only glucose. Band 1.2, therefore, is cyanidin 3,5-diglucoside.

The glycosidic pattern of anthocyanins is remarkably variable. The characterization of cyanidin 3-arabinoside-5-glucoside from flowers of Red Wing azaleas represents a new glycosidic class and extends the total number known to eighteen.[7] Other anthocyanins found in these tissues were cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-arabinoside and cyanidin 3,5-diglucoside.

EXPERIMENTAL

Extraction and Isolation

Fresh Red Wing azalea flowers were dried at 50° in a forced-draft oven and then ground to pass a 40-mesh screen. The ground tissue was extracted with light petrol (boiling range 38–49°) followed by ethyl ether. Anthocyanins were extracted by blending 40 g of the ground tissue for 5 min with 1 l. of cold methanolic 1 % HCl. The extract was filtered and the volume of the filtrate was concentrated under reduced pressure at 40° to approximately 100 ml. The anthocyanins were precipitated with 3 vol. of ethyl ether and the precipitate dissolved in a minimum volume of 0·1 N HCl. The anthocyanin solution was then placed on a column of powdered nylon[5] (4 × 40 cm). The column was then washed with 0·1 N HCl until the leading anthocyanin band had moved the length of the column. The anthocyanins were then eluted with methanol–water–HCl (100:400:1 v/v). The anthocyanins passed through the powdered nylon column resolved into two bands. Each band was further purified by preparative thin-layer chromatography.[12] The solvents used were BAW (butan-1-ol–acetic acid–water, 6:1:2 v/v), 10 % acetic acid, and the upper phase of butan-1-ol–2 N HCl. The R_f values of the purified anthocyanins were determined by thin-layer chromatography.[13–15]

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Acid Hydrolysis

The purified anthocyanin was hydrolyzed with 1 ml 1 N HCl at 100° for 1 hr. The anthocyanidin fraction was extracted with iso-amyl alcohol and the aqueous phase examined for sugars. The aqueous phase containing the sugars was deacidified with di-*n*-octylmethylamine[16] and then concentrated to approximately 0·1 ml under reduced pressure at 40°.

Identification was made by co-chromatography on paper with authentic sugars. The sugars were located on the air-dried chromatogram by momentarily dipping them in aniline hydrogen phthalate,[17] removing the excess by blotting, and then heating them at 100° for 5 min. Determination of aglycone sugar ratios was performed as described by Harborne[18] and Pridham.[19]

Identification of anthocyanidin was made by co-chromatography with authentic compounds and spectral characteristics.

Oxidation with Hydrogen Peroxide

Anthocyanins were oxidized with H_2O_2 for the identification of sugars at the C-3-position by the procedure described by Chandler and Harper.[11]

Absorption Spectra

Ultra-violet and visible absorption spectra were determined with a Carey 15 spectrophotometer in methanolic 0·01% HCl (w/v). Spectral shifts in the presence of aluminum were measured by the addition of 0·5 ml of a 1% solution of $AlCl_3.6H_2O$ in methanol to 2·5 ml of the anthocyanin or anthocyanidin solution. The solutions were allowed to stand for 30 min before the spectra were determined.

Preparative Paper Chromatography

Chromatograms were prepared by streaking a methanolic 1% HCl solution of band 1 in a band, approximately 1 cm wide, across the narrow width of Whatman 3 MM paper (46 × 57 cm). The papers were irrigated for 72 hr in a chromatographic cabinet by the descending method, in BAW. The resolved two bands were cut out and eluted with 1% HCl in 50% aq. methanol.

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