Phytochemistry, Vol. 22, No. 8, pp. 1735–1740, 1983.
Printed in Great Britain.

0031-9422/83$3.00 + 0.00
Pergamon Press Ltd.

A CYANIDIN GLYCOSIDE GIVING SCARLET COLORATION IN PLANTS OF THE BROMELIACEAE*

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(*Received* 18 *January* 1983)

Key Word Index—*Aechmea; Ananas; Billbergia*; Bromeliaceae; anthocyanins; plant colour; chemotaxonomy; cyanidin 3,5,3′-triglucoside; cyanidin 3-rutinoside-3′-glucoside; cyanidin 3,3′-diglucoside.

Abstract—A survey of leaf, bract, flower and fruit pigments in 34 taxa from 16 genera of the Bromeliaceae has shown that the dominant anthocyanin is a new glycoside of cyanidin, the 3,5,3′-triglucoside. This pigment is particularly abundant in the leaves and bracts of the subfamily Bromelioideae, occurring, for example in the pineapple, *Ananas comosus*. Three other novel cyanidin glycosides, the 3,3′-diglucoside, the 3-rutinoside-3′-glucoside and the 3-rutinoside-5,3′-diglucoside, occur in the family; the latter pigment has only been tentatively identified. The presence of a glucose residue in the 3′-position causes a hypsochromic shift in the visible spectrum and all these novel anthocyanins are scarlet instead of crimson in colour. Their production appears to be the result of natural selection for floral colouration preferred by bird pollinators. The 3,5-diglucosides of cyanidin, peonidin and malvidin regularly accompany these new pigments in this family. The orange-red flowers of *Pitcairnia sprucei* and *P. integrifolia* are distinctive in having pelargonidin 3-rutinoside, whereas most of the species with mauve or purple flowers have malvidin 3,5-diglucoside. Pelargonidin 3,5-diglucoside was identified in the bracts of *Billbergia buchholtzii* and *Tillandsia flabellata*. Overall, the anthocyanin pattern in the family is quite distinctive; however, the presence of the rare cyanidin 3′-glycosides indicates an affinity with the Commelinaceae, since the 3,7,3′-triglucoside of cyanidin in acylated form is widespread in the latter family.

INTRODUCTION

The Bromeliaceae is a large, predominantly tropical family with some 46 genera and more than 1900 species, all restricted to the New World except for one West African species, *Pitcairnia feliciana*. Many species have large, showy inflorescences and are insect or bird pollinated. Economically, the most useful member is the pineapple plant, *Ananas comosus*, but there are also many valuable ornamentals. Taxonomically, the family occupies a somewhat isolated position, although there are some morphological affinities with the Commelinaceae and Zingiberaceae [1–3]. In the latest treatment of the Monocotyledoneae, it is placed in the superorder Liliiflorae, near Haemodoraceae [4].

The present anthocyanin survey follows a recent investigation of anthocyanins in the Commelinaceae [5] and forms part of a larger survey of monocotyledonous families for their flavonoids [5–9]. No previous work has been carried out on the bromeliad anthocyanins, but the leaf flavonoids have been examined in some detail. The family appears to be unique amongst the monocotyledons in the variety and frequency of occurrence of flavones and flavonols with extra hydroxylation or methoxylation at the 6-position [6]. We have now surveyed 34 taxa representing 16 genera and report here the discovery of four new cyanidin glycosides in these plants.

*Dedicated to Professor Edgar Lederer, on the occasion of his 75th birthday on 5 June 1983.

RESULTS

Cyanic colour is particularly abundant within the Bromeliaceae, giving in many plants purple-blue to orange-red flowers, bright scarlet to orange-red bracts and also brownish-red to red leaves. Other tissues, including stamens and fruits, may also be coloured. The results of surveying the anthocyanins in 32 species and two cultivars are indicated in Table 1. The most striking feature is the frequency of cyanidin derivatives in the inflorescences and leaves, in spite of the fact that pelargonidin-like colours dominate in these tissues. The reason for this apparent discrepancy came when a new glycosidic type of cyanidin was discovered in some 60% of the sample. This new glycosidic type, the 3,5,3′-triglucoside, is characteristically present in the bracts and leaves, being particularly dominant in the subfamily Bromelioideae. Moreover, two other new 3′-glucosides, namely cyanidin 3-rutinoside-3′-glucoside and cyanidin 3,3′-diglucoside, occur in the red to purple-red leaves of *Aechmea weilbachii* var. *leodiensis* and of *Nidularium innocentii* var. *innocentii*. A small amount of a fourth new cyanidin glycoside was isolated and tentatively identified from R_f data and the results of acid hydrolysis as cyanidin 3-rutinoside-5,3′-diglucoside; this pigment occurs in the bracts of *Billbergia buchholtzii*.

Cyanidin 3,5,3′-triglucoside and 3-rutinoside-5,3′-diglucoside show a similar orange fluorescence in UV light as pelargonidin 3,5-diglucoside and all four novel cyanidin glycosides are bright scarlet in daylight instead of the deep crimson of other cyanidin glycosides. These four pigments were identified by standard procedures (see Experimental) and this represents the first report of their natural occurrence. Cyanidin 3,3′-diglucoside has been

Table 1. The distribution of anthocyanins in the Bromeliaceae

Subfamily, genus, species (Kew accession number)		Anthocyanins			
		Pelargonidin Glycosides	Cyanidin Glycosides	Peonidin Glycosides	Malvidin Glycosides
Bromelioideae					
Aechmea tillandsioides (Mart.)	orange-red B	—	3G5G3′G	—	—
Baker var. *kienastii*	scarlet C	—	3G5G3′G	—	—
(326-75.03107)	dark blue FR	—	3G5G	—	—
A. bracteata (Sw.)	orange-red B	—	3G5G3′G, 3G5G	—	—
Griseb. (590-67.59001)	dark blue FR	—	—	—	(Dp glyc.)
A. recurvata (Klotsch) L. B. Smith	scarlet B	—	3G5G3′G, 3G5G	3G5G	3G5G
var. *recurvata* (033-72.0032)	purple F	—	—	—	3G5G
A. weilbachii Didr. var. *leodiensis* André (262-71.02399)	scarlet L	—	3G5G3′G, 3RG3′G, 3G3′G	—	—
Ananas comosus (L.) Merrill (402-71.03825)	bright red L	—	3G5G3′G, 3G5G	3G5G	—
Billbergia buchholtzii Mez	red-green L	—	3G5G3′G, 3G5G	—	—
	orange-red B	3G5G,3RG5G	3G5G3′G,3G5G,3RG5G3′G	—	—
	purple F	—	—	—	3G5G
Billbergia cv. *fantasia* (620-58.62007)	red-green L	—	3G5G3′G,3G5G	—	—
Canistrum cyathiforme (Vellozo) Mez (019-72.00200)	red L	—	3G5G3′G,3G5G	—	3G5G(?)
Fascicularia kirchoffiana	scarlet L	—	3G5G3′G	—	—
Neoregelia cruenta (R. Graham) L. B. Smith (004-72.00034)	red-green L	—	3G5G,3G	3G5G	—
N. carolinae (Beer) L. B. Smith var. *carolinae* (500-65.50031)	pink-red L	—	3G5G,3G	3G5G	3G5G
N. eleutheropetala (Ule) L. B. Smith (024-72.00254)	brown-red L	—	3G5G3′G,3G5G	3G5G	—
N. princeps (Baker) L. B. Smith (024-72.00254)	pink-red L	—	3G5G	3G5G	3G5G
Nidularium innocentii Lem.	scarlet L	—	3G5G3′G,3G5G	3G5G	—
(444-72.04017)	purple F	—	—	—	3G5G (+Dp, Pt glyc.)
N. innocentii Lem. var. *lineatum* (Mez) L. B. Smith (197-73.01785)	bright red L	—	3G5G3′G,3G5G,3F3′G,3RG3′G	—	3G
N. terminale Ule (132-73.01477)	bright red L	—	3G5G3′G,3G5G	3G5G	3G5G

Table 1. (*Contd.*)

Subfamily, genus, species (Kew accession number)		Anthocyanins: Pelargonidin Glycosides	Cyanidin Glycosides	Peonidin Glycosides	Malvidin Glycosides
Portea petropolitana (Wawra) Mez	red B	—	3G5G	3G5G	—
(518-67.51804)	purple F	—	3G5G	—	3G5G
Streptocalyx poeppigii Beer	scarlet B	—	3G5G3'G,3G5G	—	—
(118-71.01145)	scarlet L	—	3G5G3'G,3G5G	—	—
Pitcairnioideae					
Dyckia niederleinii Mez (362-66)	red green L	—	3G	—	—
Pitcairnia integrifolia Gawl (407-74.03199)	orange red F	3RG	—	—	—
P. sprucei Baker (372-76.03580)	orange red F	3RG	—	—	—
P. tabuliformis Linden (495-79.05593)	bright red L	—	3G5G3'G	3G5G	3G5G
Puya laxa L. B. Smith (198-81.02830)	red-green L	—	3G5G,3G	—	—
Tillandsioideae					
Cryptanthus bahianus L. B. Smith (0032-80.00284)	brown green L	—	3G5G	3G5G	3G5G
C. bivittatus (Hook.) Regel (620-58.62006)	pink green L	—	3G5G3'G	3G5G	3G5G
C. x *osyanthus* Witte (262-71.02408)	pink green L	—	3G5G3'G	3G5G	3G5G
C. sinuosus L. B. Smith (152-73.01482)	red green L	—	3G5G3'G,3G5G	3G5G	—
C. zonatus (Vis.) Beer forma *zonatus* (461-57.46111)	red green L	—	3G5G3'G	3G5G(?)	—
Guzmania zahnii (Hook. f.) Mez (197-73.01802)	red L	—	3G5G	3G5G	—
Tillandsia flabellata Baker	red B	3G5G	—	3G5G	—
(196-73.01743)	purple F	—	—	—	3G5G
T. monadelpha (Morren) Baker (310-73.0427)	brown-red L	—	3G5G	—	3G5G
T. tricolor Schlecht. et Cham.	red B	—	3G5G	3G5G	—
(001-69.51149)	blue-purple F	—	—	—	3G5G
Vriesea inflata (Wawra) Wawra (008-72.00204)	brown red L	—	—	3G5G	3G5G
V. platynema Gaud. (0032-72.0408)	brown-red L	—	3G	3G,3-glyc.	—

Key: L, leaf; B, bract; F, flowers; C, calyx; FR, fruit; 3G, 3-glucoside; 3RG, 3-rutinoside; 3G5G, 3,5-diglucoside; 3G3'G, 3,3'diglucoside; 3G5G3'G, 3,5,3'-triglucoside; 3RG5G, 3-rutinoside-5-glucoside; 3RG5G3'G, 3-rutinoside-5,3'-diglucoside; Dp, delphinidin.

obtained previously by partial acid hydrolysis of the acylated cyanidin 3,7,3′-triglucoside present in members of the Commelinaceae [5, 10] and indeed products of the partial hydrolysis of this latter pigment were used extensively for purposes of direct comparison with the products of partial hydrolysis of the bromeliad pigments (Table 2).

Although having very similar colour and spectral properties ($\lambda_{max}^{MeOH-HCl}$ 516–518 nm) to pelargonidin glycosides ($\lambda_{max}^{MeOH-HCl}$ 510–514 nm), these novel cyanidin glycosides have not completely replaced pelargonidin derivatives in bromeliad tissues. Thus, pelargonidin 3-rutinoside was found in orange flowers of *Pitcairnia integrifolia* and *P. sprucei*, while pelargonidin 3,5-diglucoside was detected as a minor pigment in the bracts of *Billbergia buchholtzii* and *Tillandsia flabellata*. The latter plant contained small amounts of a pelargonidin glycoside identified as pelargonidin 3-rutinoside-5-glucoside on the basis of TLC and spectral data (Table 2).

The well-known and widely distributed 3,5-diglucosides of cyanidin, peonidin and malvidin were found to accompany the above pelargonidin and cyanidin glycosides in leaves and bracts of most species surveyed (Table 1). The related 3-glucosides were occasionally detected. In the case of floral tissues, malvidin 3,5-diglucoside was often present as a major pigment in plants with purple-blue flowers, sometimes accompanied by traces of delphinidin and petunidin glycosides. As already mentioned above, the orange flowers of *Pitcairnia integrifolia* and *P. sprucei* uniquely contain pelargonidin 3-rutinoside rather than the expected 3,5-diglucoside.

Fruits were only available for examination in the case of *Achmea bracteata* and *A. tillandsioides*; the blue-black skin colours were due to delphinidin glycoside and cyanidin 3,5-diglucoside, respectively.

DISCUSSION

Anthocyanin patterns have now been explored in some 15 families within the monocotyledons [5, 11–14]. A comparison with the data available for the other families (Table 3) indicated that the Bromeliaceae is unique in having a glycosidic pattern based on 3,5,3′-trisubstitution. Thus, cyanidin 3,5,3′-triglucoside is a distinctive marker for the family, occurring in over 60% of the 34 taxa studied. It is especially common in members of the Bromelioideae, the subfamily which, in terms of leaf flavonoids [6], has the simplest pattern. The only other monocot family with B-ring substituted anthocyanins is the Commelinaceae where 3,7,3′-trisubstitution is common. There is, thus, some chemical affinity between the two families. It may be noted, however, that the Commelinaceae anthocyanins are usually acylated, whereas acylation is unknown among the bromeliad pigments.

In the angiosperms as a whole, B-ring substitution by sugar in anthocyanins is still quite rare. The tricaffeoyl and feruloylcaffeoyl esters of cyanidin and delphinidin 3,7,3′-triglucosides found in the Commelinaceae have also been reported in the Compositae [15, 16]. Additionally, a dicaffeoyldelphinidin 3,5,3′-triglucoside has been isolated from *Gentiana* (Gentianaceae) [17] while a *p*-coumaroylferuloylcaffeoyl delphinidin 3-rutinoside-5,3′,5′-triglucoside occurs in *Lobelia* (Lobeliaceae) [18]. In all these cases, however, the fact that 3′-glucosylation causes a hypsochromic shift in colour is masked because other factors, especially the presence of acylation, are involved in flower colour. These pigments have been isolated from blue flowers. Acylation of the sugar residues around the molecule not only stabilizes the flavylium cation in the aqueous environment of the cell vacuole but also produces a bathochromic shift towards longer wavelengths.

By contrast, in the Bromeliaceae, the effect of B-ring substitution can clearly be seen to be hypsochromic, because the cyanidin derivatives with 3′-glucosylation are scarlet in colour. Indeed, cyanidin 3,5,3′-triglucoside contributes to scarlet colouration in calyces (e.g. in *Aechmea tillandsioides*) but particularly in bracts (e.g. in *A. bracteata* and many other species). 3′-Glucosylation of

Table 2. R_f values and spectral properties of Bromeliaceae anthocyanins

	R_f (×100)				Absorption spectra in MeOH			
Anthocyanin	BAW	Bu–HCl	1% HCl	HOAc–HCl	Spectral max (nm)		$E_{440}/E_{visible\ max}$	+$AlCl_3$
Pelargonidin 3-rutinoside	42	24	15	42	270	514	30	—
3,5-diglucoside	35 (31)*	12 (14)*	18 (23)*	43 (45)*	270	510	25 (21)*	—
3-rutinoside-5-glucoside	27	11	43	66	270	512	19	—
Cyanidin 3-glucoside	36	13	04	15	283 (282)†	530 (530)†	24 (21)*	+
3,5-diglucoside	25 (28)*	04 (6)*	10 (16)*	30 (40)*	277 (279)†	526 (526)†	19 (13)*	+
3,3′-diglucoside	26	07	13	38	277 (279)†	519 (519)†	42 (35)†	
3-rutinoside-3′-glucoside	26	07	29	53	278	521	39	—
3,5,3′-triglucoside	21	01	33	55	277	518	19	—
3-rutinoside-5,3′-diglucoside	15	02	52	71	280	516	23	—
Peonidin 3-glucoside	47	16	06	22	282	529	27	—
3,5-diglucoside	32 (31)*	06 (10)*	13 (17)*	38 (44)*	280	526	15 (13)*	—
Delphinidin 3-glucoside	31	07	02	15	—	—	—	—
Malvidin 3-glucoside	39	09	04	20	—	—	—	—
3,5-diglucoside	30	04	08	34	276	536	19	—

Solvent key: BAW, *n*-BuOH–HOAc–H_2O (4:1:5); Bu–HCl, *n*-BuOH–2 M HCl (1:1); 1% HCl, H_2O–conc. HCl (97:3); HOAc–HCl, H_2O–HOAc–HCl (82:15:3).

*Data according to Harborne [10].

†Data according to Yoshitama and Abe [11].

Table 3. Summary of anthocyanin patterns of some monocotyledonous families

Family	Anthocyanidin present				Major glycosidic patterns				
	Pg	Cy	Dp	Methyl derivatives	3-Monoside	3-Diglucoside	Additional 5-glycosylation	Additional 3'glycosylation	Acylation
Hydrocharitaceae	−	+	−	+	+	−	−	−	−
Gramineae	(+)	+	(+)	+	+	+	−	−	−
Araceae	+	+	−	(+)	+	+	−	−	+
Lemnaceae	−	+	+	+	+	−	−	−	+
Cannaceae	−	+	−	−	+	+	−	−	−
Zingiberaceae	−	+	(+)	+	+	+	−	−	−
Musaceae	−	+	+	+	+	+	−	−	−
Marantaceae	(+)	+	+	+	+	+	(+)	−	(+)
Amaryllidaceae	+	+	−	−	+	+	−	−	−
Dioscoreaceae	−	+	+	+	−	+	+	−	+
Liliaceae	+	+	+	+	+	+	+	−	+
Iridaceae	+	+	+	+	+	+	+	−	+
Orchidaceae	+	+	+	−	+	(+)	+	−	+
Commelinaceae	−	+	+	−	+	−	+	+	+
Bromeliaceae	+	+	+	+	+	(+)	+	+	−

Data mainly from refs. [5–13].

Key: Pg, pelargonidin; Cy, cyanidin; Dp, delphinidin; methyl derivatives include peonidin, 5-methylcyanidin, petunidin and malvidin; 3-monoside, mainly 3-glucoside, occasionally 3-galactoside or 3-arabinoside; 3-diglycoside, mainly 3-rutinoside; +, very common; (+), infrequent; −, not reported.

cyanidin derivatives, thus, appears to be an alternative evolutionary pathway towards the scarlet and orange colours preferred by bird pollinators. Such colours are more commonly achieved by mutation of the gene controlling 3'-hydroxylation to give pelargonidin [19]. However, the replacement of the more common pathway by that involving 3'-glucosylation is not complete in Bromeliaceae, since pelargonidin is still present, giving an orange colour in the flowers of several species. One may assume, by analogy with the cyanidin series, that 3'-glucosylation of pelargonidin 3,5-diglucoside would produce a yellow pigment, another colour which is preferred by bird pollinators. Since yellow flower colour is common in certain bromeliad genera, it will be interesting to see if these are carotenoid-based or whether the so far undescribed pelargonidin 3,5,3'-triglucoside is present. Further work along these lines is in progress.

EXPERIMENTAL

Plant material. Leaves, bracts and flowers were collected from the living collection of the Bromeliaceae at the Royal Botanic Garden, Kew, and accession numbers are given in Table 1. The plants of *Nidularium innocentii* and *Fascicularia kirchoffiana* studied were from the University of Reading plant collection.

Anthocyanin identifications. Anthocyanins were surveyed by 1D PC and TLC (Merck Cellulose No. 5577) in the four standard solvents [20] against cyanidin 3,5-diglucoside, peonidin 3,5-diglucoside, malvidin 3,5-diglucoside, pelargonidin 3,5-diglucoside, cyanidin 3-rutinoside, pelargonidin 3-rutinoside and the 3-monoglucosides of cyanidin, pelargonidin, peonidin, delphinidin, petunidin and malvidin as markers. In the majority of taxa and where sufficient pigment was available, the anthocyanins were separated and purified by PC and identified by co-chromatography with markers, by spectral measurements and by detection of the appropriate anthocyanidin and sugars on acid hydrolysis.

Identification of cyanidin 3,3'-diglucoside from Aechmea weilbachii *var.* leodiensis. The glucoside was isolated from a 0.5% HCl–MeOH leaf tissue extract by prep. PC in BAW and TLC in 1% HCl. Acid hydrolysis gave glucose and cyanidin. The identity of the glycoside was confirmed by co-PC and TLC in four solvents with an authentic pigment of cyanidin 3,3'-diglucoside obtained by partial hydrolysis of the *Zebrina* anthocyanin, and also a comparison with UV and visible spectral data: $\lambda_{max}^{1\% HCl\text{-}MeOH}$ nm: 279, 510; $+AlCl_3$ 0, $E_{440}/E_{visible\ max} = 42\%$. Partial hydrolysis gave cyanidin, cyanidin 3-glucoside and cyanidin 3'-glucoside.

Identification of cyanidin 3-rutinoside-3'-glucoside from Nidularium innocentii *var.* lineatum. The glycoside was isolated and purified as above. Acid hydrolysis gave glucose, rhamnose and cyanidin. The λ_{max} of this pigment also did not shift on the addition of $AlCl_3$. R_f and absorption data are given in Table 2. Partial hydrolysis gave cyanidin, cyanidin 3-glucoside, cyanidin 3'-glucoside, cyanidin 3-rutinoside and cyanidin 3,3'-diglucoside. This glycoside was further confirmed by H_2O_2 oxidation and direct chromatographic comparison of the rutinose formed with authentic disaccharide.

Identification of cyanidin 3,5,3'-triglucoside from Nidularium innocentii. The anthocyanin mixture was extracted and purified by the same procedure as described in ref. [21]. This glycoside was isolated and purified from the anthocyanin mixture on cellulose TLC plates in *n*-BuOH–HCl–H_2O (7:2:5). FAB MS established the MW as 773 ($C_{33}H_{41}O_{21}$ requires 773). Acid hydrolysis gave glucose and cyanidin. The λ_{max} of this pigment did not shift on the addition of $AlCl_3$, indicating substitution in the 3'- or 4'-positions. R_f and absorption data for this pigment are given in Table 2. Partial hydrolysis gave cyanidin, cyanidin 3-glucoside, cyanidin 5-glucoside, cyanidin 3'-glucoside, cyanidin 3,3'-diglucoside and cyanidin 3,5-diglucoside. This structure was confirmed by H_2O_2 oxidation, which gave only glucose as the product.

Cyanidin 3-rutinoside-5,3′-diglucoside from Billbergia buchholtzii. Five anthocyanins were isolated from this plant, and of them were identified as pelargonidin 3,5-diglucoside, pelargonidin 3-rutinoside-5-glucoside, cyanidin 3,5-diglucoside and cyanidin 3,5,3′-triglucoside. Due to the small amount of plant material available, it was impossible to purify the fifth pigment sufficiently to allow its complete characterization. However, R_f values, spectral data and acid hydrolysis products suggest cyanidin 3-rutinoside-5,3′-diglucoside.

Acknowledgements—We are grateful to the Director of the Royal Botanic Gardens, Kew, for provision of plant material, and also to Dr. C. A. Williams and Mr. S. J. Mayo for their help and encouragement. We thank Mr. D. Philcox of Kew for assistance with taxonomic nomenclature and Dr. C. Tamura, Sankyo Co. Ltd., for the MS measurement on the Jeol GC/MS-KT (34) instrument.

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