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COMPARATIVE BIOCHEMISTRY OF FLAVONOIDS—II.

3-DESOXYANTHOCYANINS AND THEIR SYSTEMATIC DISTRIBUTION IN FERNS AND GESNERADS*

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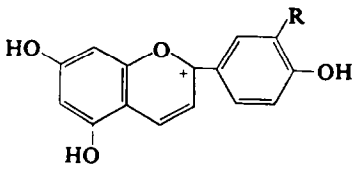
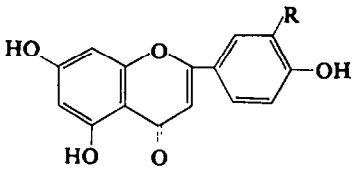
Abstract—The red pigments in ferns were identified for the first time as 3-desoxyanthocyanins. Apigeninidin and luteolinidin were found, mainly as the 5-glucosides but also in several other combined forms, in the juvenile fronds of eight species. They also occurred in the red sori of *Dryopteris erythrosora*. The earlier report of common anthocyanins in *Dryopteris* could not be confirmed, but pelargonidin and cyanidin derivatives were definitely identified in *Davallia divaricata*. The distribution of 3-desoxyanthocyanins in the Gesneriaceae, the family from which they were first isolated, was also studied. They were found in 18 of 21 species in the sub-family Gesnerioideae but were absent from 25 species in the sub-family Cyrtandroideae. These results support Burtt's recent re-classification of the two sub-families on the basis of presence or absence of anisocotyly and geographical distribution but do not fit Fritsch's division of the family based on the position of the ovary. A new 3-desoxyanthocyanidin, columnidin, was isolated from *Columnea* × *banksii* and provisionally identified as 5,7,8,3′,4′-pentahydroxyflavylium. While common anthocyanins (e.g. malvidin 5-glucoside-3-rutinoside and pelargonidin-3-rutinoside) also occur in the Gesneriaceae, flavonols are absent. The flavones apigenin, luteolin and diosmetin were identified in nine species. It is suggested that 3-desoxyanthocyanin production is basically a primitive plant character but that these pigments are synthesized in the highly advanced Gesneriaceae in response to natural selection for a bright orange-red flower colour.

INTRODUCTION

3-DESOXYANTHOCYANINS, e.g. luteolinidin (I), are of considerable phytochemical interest, since, although they are related to the flavones as the common anthocyanidins are related to the flavonols, they are themselves of rare occurrence. Thus, apigenin (III) and luteolin (IV) were isolated from plants before 1900[1] and have since been found to occur in the petals and leaves of many higher plants.[1–3] By contrast, apigeninidin (II) as the 5-glucoside (gesnerin),



(I) R = OH; Luteolinidin
(II) R = H; Apigeninidin



(III) R = H; Apigenin
(IV) R = OH; Luteolin

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[1] W. KARRER, *Konstitution und vorkommen der organischen Pflanzenstoffe*, p. 673. Birkhauser Verlag, Berlin (1958).
[2] E. C. BATE-SMITH, *J. Linnean Soc. London (Botany)* **58**, 95 (1962).
[3] J. B. HARBORNE and N. W. SIMMONDS, *Biochemistry of Phenolic Compounds*, p. 71. Academic Press, London (1964).

was first isolated from the petals of several Gesneriaceae by Robinson *et al.* in 1934.[4] The luteolin analogue, luteolinidin, was not found in the Gesneriaceae until 1960[5]; the 3',4',5'-trihydroxy analogue, tricetinidin, was discovered in tea, *Camellia sinensis*, in 1958.[6] The only other reported occurrences of 3-desoxyanthocyanins are in the monocotyledon *Sorghum vulgare* (Poaceae),[7] in two dicotyledons *Arrabidaea chica* (Bignoniaceae)[8] and *Chirantho-dendron pentadactylon* (Sterculiaceae),[9] and in the moss, *Bryum cryophyllum*.[10]

The recent discovery of luteolinidin in mosses suggested that 3-desoxyanthocyanins might also occur in ferns. Some preliminary work had, in fact, been carried out on the red pigmentation in young fern fronds. Thus, Price *et al.* in 1938[11] reported that unusual flavylium salts, resembling 6-hydroxycyanidin or 6-hydroxypelargonidin in their colour properties, occurred in eight ferns. These authors also reported an ordinary anthocyanin, an acylated pelargonidin dimonoside, in *Davallia divaricata* and later Hayashi and Abe,[12] during a chromatographic survey of land plants, described *Dryopteris erythrosora* as containing two cyanidin glycosides. The fern pigments have now been reinvestigated[13] and found to be 3-desoxyanthocyanins.

The distribution of 3-desoxyanthocyanins in the Gesneriaceae has also been studied. This was carried out partly to search for new 3-desoxyanthocyanidins (particularly for *O*-methylated derivatives) and partly in order to see if there was any relationship to the taxonomy of the group. The result of these surveys are presented in this paper.

RESULTS

Identification of 3-Desoxyanthocyanins

3-Desoxyanthocyanins and their corresponding aglycones are readily distinguished from the common 3-hydroxylated anthocyanidins by means of (*a*) their distinctive colour, chromatographic and spectral properties and (*b*) their greater stability to oxidation and (*c*) the failure of the aglycone to fade on chromatograms developed in solvents lacking mineral acid. There is, therefore, no difficulty in identifying these pigments on a micro-scale. The present work has, of necessity, been carried out with limited amounts of plant material but, in all cases, unknown pigments have been directly compared in as many ways as possible with authentic samples. In view of the earlier suggestions of Price *et al.*[11] that the fern pigments might be 6-hydroxylated anthocyanidins, it seemed important to compare them with such compounds. Indeed, it is well known that introduction of a 6-hydroxyl group and removal of a 3-hydroxyl group both have a hypsochromic effect on anthocyanin spectra in the visible region. 6-Hydroxypelargonidin and 6-hydroxycyanidin were synthesized by standard procedures and a comparison of their properties with those of apigeninidin and luteolinidin clearly shows they are different. 6-Hydroxypelargonidin and luteolinidin do have almost identical $\lambda_{max.}$ in the visible region but their R_f values are quite distinct (Table 1).

[4] G. M. ROBINSON, R. ROBINSON and A. R. TODD, *J. Chem. Soc.* 809 (1934).
[5] J. B. HARBORNE, *Chem. & Ind. (London)* 229 (1960).
[6] E. A. H. ROBERTS and D. M. WILLIAMS, *J. Sci. Food Agr.* **9**, 217 (1958).
[7] H. A. STAFFORD, *Plant Physiol.* **40**, 130 (1965).
[8] E. CHAPMAN, A. G. PERKINS and R. ROBINSON, *J. Chem. Soc.* 3015 (1927).
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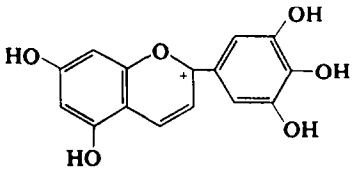
[10] G. BENDZ, O. MARTENSSON and L. TERENIUS, *Acta Chem. Scand.* **16**, 1183 (1962).
[11] J. R. PRICE, V. C. STURGESS and R. ROBINSON, *Nature* **142**, 356 (1938).
[12] K. HAYASHI and Y. ABE, *Botan. Mag. Tokyo* **68**, 299 (1955).
[13] J. B. HARBORNE, *Nature* **207**, 984 (1965).

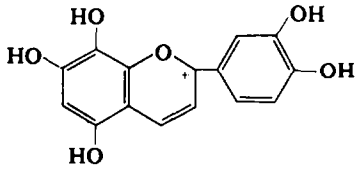
TABLE 1. PROPERTIES OF 3-DESOXYANTHOCYANIDINS AND OF 6-HYDROXYLATED ANTHOCYANIDINS

		R_f value in solvent				Spectral maxima (mμ)			
	Colour	Forestal	Formic	BAW	EtAc–HCO_2H	MeOH–HCl	440/max ratio (%)	MeOH–NaOH	MeOH–$AlCl_3$
3-Desoxyanthocyanidins									
Apigeninidin	Yellow	0·78	0·75	0·78	0·65	277,476	55	533	476
Luteolinidin	Orange	0·61	0·64	0·42	0·43	279,496	45	565	544
Luteolinidin 3′-methyl ether	Orange	0·77	0·73	0·71	0·56	277,493	56	557	493
Tricetinidin	Orange-red	0·46	0·47	0·38	0·21	281,513	22	564 (fades slowly)	553
Tricinidin	Orange-red	0·78	0·83	0·43	0·50	279,509	32	587	509
Columnidin	Orange-red	0·54	0·31	0·54	0·23	275,511	23	560	—
6-Hydroxyanthocyanidins									
6-hydroxypelargonidin	Orange	0·57	0·56	0·58	0·24	290,497	37	unstable	—
6-hydroxycyanidin	Red	0·30	0·32	0·39	0·21	283,518	25	unstable	—

Columnin, the glucoside of a new 3-desoxyanthocyanidin, was isolated from petals of *Columnea* × *banksii*; it also occurred in most other *Columnea* species examined, as well as in leaves of plants of related genera (see later). Hydrolysis gave a new aglycone, columnidin, which from its general stability and other properties (Table 1) clearly lacks a 3-hydroxyl constituent. Columnidin has a molecular weight of 287, indicating that it is a pentahydroxyflavylium. The only 3-desoxyanthocyanidin of this type known is tricetinidin (5,7,3′,4′,5′-pentahydroxyflavylium) (V). Columnidin is similar in spectral properties but the two compounds have different R_f values (see Table 1). On reductive cleavage with alkali and



(V) Tricetinidin



(VI) Columnidin

sodium amalgam.[14] columnidin yields 1,2,3-trihydroxybenzene and 3,4-dihydroxyphenylpropionic acid as the main products. Thus, it is either 5,7,8,3′,4′- or 5,6,7,3′,4′-pentahydroxyflavylium. Of these two structures, the spectral data strongly favour the former (VI) but further work is required to confirm this.

3-Desoxyanthocyanins in Ferns

Little is known about the distribution of red pigmentation in ferns, so that the present studies were limited to material already known to synthesize anthocyanin in juvenile fronds. Fortunately, three of the eight species examined by Price *et al.*,[11] i.e. *Adiantum veitchianum*, *Blechnum brasiliense* and *Osmunda regalis*, were already available and all these, when grown in the glasshouse, produced anthocyanin. A number of related species were grown at the same time and four of these were also pigmented. Another species, *Adiantum pedatum*, was obtained, in which, exceptionally, the reddish pigmentation was retained in the mature plant.

Fresh fronds were collected, extracted and the pigments present separated by paper chromatography and examined by the standard procedures. All but one of the pigments from the eight species (Table 2) gave luteolinidin (I) or apigeninidin (II) on hydrolysis. The only sugar detected after hydrolysis was glucose and the 5-glucosides of luteolinidin and apigeninidin were identified in six fern species, by direct comparison with authentic pigments isolated from the gesnerad *Rechsteineria cardinalis*. Seven other glycosides were present (Table 2), but not in sufficient amount for complete identification. They were similar, except in R_f value, to the two known glucosides and three gave glucose as the only sugar on hydrolysis. Their spectral properties were identical with those of the 5-glucosides, indicating that they all bore a substituent on the 5-hydroxyl group. However, the fact that these unidentified pigments had higher R_f values in all solvent systems, when compared with the 5-glucosides, suggests that they may be more complex in structure. Thus, none of the luteolinidin derivatives is identical with the 5-diglucoside isolated from *Bryum*[10] which, in comparison with luteolinidin 5-glucoside, has higher R_f values in aqueous solvents but lower R_f values in alcoholic solvents.

Dryopteris erythrosora, reported by Hayashi and Abe to be pigmented by two cyanidin glycosides,[12] contained only 3-desoxyanthocyanins: an apigeninidin glycoside, a luteolinidin

[14] J. B. HARBORNE and H. M. HURST, Unpublished procedure.

TABLE 2. DISTRIBUTION AND PROPERTIES OF 3-DESOXYANTHOCYANINS IN FERNS

Distribution

Fern species	Desoxyanthocyanins present	Anthocyanidins formed* from glycosidic pigments	Anthocyanidins formed* from leucoanthocyanidins
Adiantum veitchianum	Gesnerin, luteolinidin 5-glucoside, Adiantum-1 and Adiantum-2	Ad, Lt	Cy
Adiantum pedatum cv. "Klondyke"	Gesnerin and luteolinidin 5-glucoside	Ad, Lt	Dp, Cy
Blechnum brasiliense var. *corcovadense*	Gesnerin and luteolinidin 5-glucoside	Ad, Lt	Cy
Dryopteris erythrosora	Dryopteris-1, Dryopteris-2 and Dryopteris-3	Ad, Lt	Dp, Cy
Osmunda regalis (Royal Fern)	Pteris-1	Lt	Cy
Pteris longipinnula	Gesnerin, luteolinidin 5-glucoside and Pteris-1	Ad, Lt	Pg
Pteris quadriaurita	Luteolinidin 5-glucoside, Pteris-1 and Pteris-2	Lt	Pg
Pteris vittata	Luteolinidin 5-glucoside	Lt	Dp, Cy

Properties

Pigments	R_f values† on Whatman No. 1 paper in BAW	BuHCl	1% HCl	HOAc–HCl	λ_{max} (mμ) in MeOH–HCl	440/max ratio	MeOH–NaOH	MeOH–$AlCl_3$
Apigeninidin glycosides								
gesnerin (5-glucoside)	0·41	0·38	0·22	0·55	274, 477	45%	—	477
Adiantum-1	0·47	0·52	0·31	0·64				
Dryopteris-1	0·41	0·41	0·53	0·77				
Luteolinidin glycosides								
5-glucoside	0·31	0·27	0·13	0·40	277, 499	23%	585	545
Adiantum-2	0·36	0·36	0·15	0·46				
Dryopteris-2	0·31	0·32	0·44	0·70				
Pteris-1	0·42	0·66	0·30	0·60				
Pteris-2	0·48	0·71	0·38	0·70				
Tricetinidin (?) glycoside								
Dryopteris-3	0·26	0·24	0·30	0·63	514	—	—	560

* Abbreviations: Ad, apigeninidin; Lt, luteolinidin; Dp, delphinidin; Cy, cyanidin; Pg, pelargonidin.

† For solvent abbreviations, see experimental. Colours of apigeninidin glycosides on paper, yellow (visible), fluorescent yellow (u.v.), scarlet (u.v. + NH_3); of luteolinidin glycosides, orange (visible), bright orange (u.v.), crimson (u.v. + NH_3). Dryopteris-3 was similar to luteolinidin derivatives but deeper in colour.

glycoside and traces of a third desoxyanthocyanin, dryopteris-3, which is probably a tricetinidin glucoside. The red sori of this plant also contained these pigments. Direct acid hydrolysis of the fronds, however, yielded some cyanidin, so that the original mis-identification may have been due to confusing anthocyanidin produced from leucocyanidin with that produced from the naturally-occurring glycosides. However, the earlier report[11] of an acylated pelargonidin glycoside in *Davallia divaricata* was confirmed. The main pigment present was identified as monardein (pelargonidin 3-*p*-coumaroylglucoside-5-glucoside) and some of the related cyanidin derivative was also present.

Although 3-desoxyanthocyanins were found in all but one of the ferns studied, normal leucoanthocyanidins were detected in all species (Table 2). In a study of five ferns, Bate-Smith[15] reported the presence of leucocyanidin and leucodelphinidin, and these compounds were found regularly in the present survey. In addition, leucopelargonidin was detected in two species, the first report of this substance in the Pteridophyta.

Distribution of 3-Desoxyanthocyanins in the Gesneriaceae

Although the family Gesneriaceae are rich in ornamental plants, their anthocyanins have been little studied. Apart from two or three species found by Robinson *et al.*[4] to contain gesnerin (apigeninidin-5-glucoside), the only other plants to have been examined in detail are *Streptocarpus* species and cultivars, which contain a series of glycosides derived from the six common anthocyanidins.[16, 17] The pigments of this family were therefore surveyed in order to determine the distribution of 3-desoxyanthocyanins and to seek 3-desoxyanthocyanins of novel structures. This survey would have been impossible without the help of B. L. Burtt, Royal Botanic Garden, Edinburgh, who very kindly put leaves and petals of many authentically identified gesnerads at the author's disposal. The results are collected in Table 3.

The plants are arranged (Table 3) according to Burtt's recent reclassification[18] of the family into sub-families on the basis of geographical distribution and the presence or absence of anisocotyly (the unequal development of the cotyledons). While isocotylous New World species contain 3-desoxyanthocyanins with high frequency (18 of 21 species examined), anisocotylous Old World species (25 species examined) have only the normal type of anthocyanin. This difference in distribution pattern between the sub-families is highly significant although the sample so far studied (under 1 per cent of the species but 22 per cent of the genera) is small.

The results of the survey thus support Burtt's reclassification of the family. For example, the tribe Columneae, which includes the genus *Columnea*, was placed by Fritsch[19] into the sub-family Cyrtandroideae because of the superior ovary. Burtt removes it to the sub-family Gesnerioideae and the presence of the new desoxyanthocyanidin, columnidin, in nine species in the tribe and of luteolinidin-5-glucoside in one species provides chemotaxonomic support for the rearrangement.

It should be noted that none of the three species shown by Robinson *et al.* to contain gesnerin still bears the same name. *Gesnera cardinalis* Hort. is now *Rechsteineria cardinalis* Lehm., *Isoloma hirsutum* Hort. is *Kohleria eriantha* Benth. and *Gesnera fulgens* is probably a *Smithiantha* species (C. V. Morton, personal communication).

Normal anthocyanins are found in both the Cyrtandroideae and the Gesnerioideae (Table 3). Two glycosidic patterns are common, 3-rutinoside and 5-glucoside-3-rutinoside; and malvidin 5-glucoside-3-rutinoside occurs in several species from both sub-families. However, two rarer pigments, the 3-sambubiosides of pelargonidin and cyanidin, have been found so far in two genera of the Cyrtandroideae (*Aeschynanthus* and *Streptocarpus*) and may possibly be confined to the sub-family.

[15] E. C. BATE-SMITH, *Biochem. J.* **58**, 122 (1954).
[16] W. J. C. LAWRENCE and V. C. STURGESS, *Heredity* **11**, 303 (1957).
[17] J. B. HARBORNE, *Phytochem.* **2**, 85 (1963).
[18] B. L. BURTT, *Notes Roy. Botan. Garden Edinb.* **24**, 205 (1962).
[19] K. FRITSCH, In *Die Naturliche Pflanzenfamilien* (Edited by ENGLER and PRANTL), IV (3B), p. 133 (1893–4).

TABLE 3. ANTHOCYANIN DISTRIBUTION IN THE GESNERIACEAE

Tribe, genus and species	Source	Petal anthocyanins*	Leaf anthocyanins
NEW WORLD SPECIES (sub-family GESNERIOIDEAE)			
Columneae			
Alloplectus vittatus	C.2855	Lt 5-glucoside†	Columnin†
Columnea affinis	C.3684	New type†	—
Columnea × *banksii*	J.I.	Columnin†	Columnin†
Columnea kucyniakii	C.3722	None	Columnin†
Columnea microphylla	J.I.	Columnin†	Columnin†
Columnea cv. "Stavanger"	J.I.	Columnin†	Columnin†
Columnea teuscheri	J.I.	Columnin†	Green
Episcia reptans	Kew	Pg 3-rutinoside	Columnin†
Nautilocalyx lynchii	C.3796	—	Columnin†
Gloxinieae			
Achimenes cvs.	J.I.	Pg or Mv 5-glucoside-3-rutinoside	Columnin†
Koellikeria erinoides	C.4271	—	Columnin (stem)†
Smithiantha cv. "Orange King"	J.I.	Gesnerin†	Pg glycoside
Kohlerieae			
Kohleria eriantha	J.I.	Gesnerin and Pg 3-rutinoside†	Lt 5-glucoside† (also in
× *Kohleria*	J.I.	Mv 5-glucoside-3-rutinoside	Columnin† sepal)
Gesnerieae			
Gesneria cuneifolia	Kew	Gesnerin Lt 5-glucoside†	—
Gesneria ventricosa	Kew	Pg 3-rutinoside	—
Sinningieae			
Rechsteineria cardinalis	J.I.	Gesnerin and Lt 5-glucoside†	Green
Rechsteineria macropoda	J.I.	Gesnerin†	Green
Sinningia speciosa	Kew	Mv 3-rutinoside	—
Sinningia gloriosa	J.I.	Pg and Cy 3-rutinoside	—
Sinningia barbata	C.1589	None	Columnin†
OLD WORLD SPECIES (sub-family CYRTANDRIODEAE)			
Trichosporeae			
Aeschynanthus obconicus	C.4516	Pg 3-sambubioside	Green
Aeschynanthus parvifolius	J.I.	Pg 3-sambubioside	Cy 3-sambubioside (sepal)
Dichrotrichum sp.	C.4045	Pg 5-glucoside-3-rutinoside	Green
Didymocarpeae			
Chirita lacunosa	C.4283	Mv glycosides	Green
Boea hygroscopica	C.3769	Dp and Cy glycosides	Green
Dichiloboea speciosa	C.4081	Dp, Pt and Mv glycosides	Green
Ornithoboea wildeana	C.3977	Pt and Mv glycosides	Green
Saintpaulia ionantha	J.I.	Mv 5-glucoside-3-rutinoside	Cy 3-sambubioside
Streptocarpus 15 spp.‡	J.I.	Mv 5-glucoside-3-rutinoside	Cy 3-sambubioside
Streptocarpus cyanandrus	C.3674	Cy 3-sambubioside	Cy 3-sambubioside
Streptocarpus dunnii	C.4515	Cy 3-sambubioside	Cy 3-sambubioside

* Abbreviations: Pg, pelargonidin; Lt, luteolinidin; Cy, cyanidin; Dp, delphinidin; Pt, petunidin; Mv, malvidin. A dash indicates that plant material was not available for examination.

† Denotes 3-desoxyanthocyanin.

‡ The species are: *cyaneus, daviesii, caulescens, gardeni, grandis, galpinii, insignis, polyanthus, gracilis, johannis, michelmorei, polackii, haygarthii, rexii* and *wendlandii*. They were all examined by Lawrence and Sturgess (reference 16) by the distribution tests, and approximately half of them by the author using chromatographic methods.

Other Flavonoids in the Gesneriaceae

In connection with the anthocyanin survey, the other flavonoids present in the family were also examined. Flavones were found to be widely distributed, chalcones or aurones occurred in a few species but flavonols were completely absent.

Flavones were found in all of twelve species chosen at random (Table 4), indicating that they are probably widely distributed. While luteolin (IV) and apigenin (III) were found in

TABLE 4. FLAVONES, CHALCONES AND AURONES IN THE GESNERIACEAE

Genus and species	Flavones present*	Chalcones or aurones present†
Gesnerioideae		
Achimenes cv.	Luteolin and apigenin (L,F)	None
Columnea × *banksii*	Luteolin and diosmetin (L)	None
Columnea microphylla		
Columnea cv. "Stavanger"		
Columnea teuscheri	Apigenin (L)	None
Kohleria eriantha	Luteolin and apigenin (L,F)	None
Rechsteineria cardinalis	Luteolin and apigenin (L,F)	None
Rechsteineria macropoda	Luteolin and apigenin (F)	None
Smithiantha cv. "Orange King"	Luteolin and apigenin (L,F)	None
Cyrtandroideae		
Aeschynanthus parvifolius	Unidentified (L)	Chalcononaringenin (F)
Boea hygroscopica	Not studied	Chalcone? (F)
Chirita micromusa	Not studied	Aureusidin (F)
Didymocarpus pedicellatus	Not studied	Pedicin, pedicellin (L)
Petrocosmea kerrii	Unidentified (L)	Aureusidin (F)
Streptocarpus spp.	Luteolin and apigenin (L,F)	Chalcones? (F)

* Present as glycosides. Luteolin 7-glucoside was identified in *Rechsteineria cardinalis* petal. A diosmetin 7-glucoside was identified in flower of *Columnea* × *banksii* (see Experimental). L = leaf; F = flower.

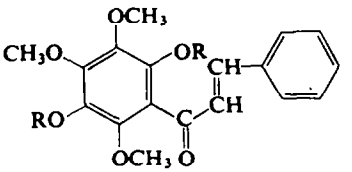
† Present mainly as glycosides. Chalcononaringenin occurs as the 6′-glucoside (isosalipurposide). Aureusidin occurs as the 4-glucoside (cernuoside).

most species, the rarer methylated flavone, diosmetin (luteolin 4′-*O*-methyl ether), was also present. The latter was positively identified in flowers of *Columnea* × *banksii* but a chromatographic survey indicated that methylated flavones may be present in other species in the sub-family Gesnerioideae.

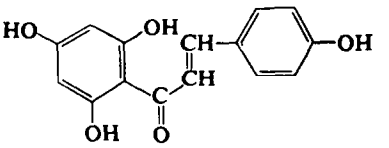
The distribution of chalcones and aurones are also of potential systematic interest, since they have been found so far only in members of the Cyrtandroideae. The chalcones, pedicin (VII) and pedicellin (VIII) were earlier isolated from leaves of *Didymocarpus pedicellata*.[20] More recently, chalcononaringenin (IX) has been identified in *Aeschynanthus parvifolius* and the aurone, cernuoside (X), in *Chirita micromusa*.[21] During the present survey, cernuoside has been found in petals of *Petrocosmea kerrii* and a chalcone or aurone has been detected in petals of *Boea hygroscopica*. It also seems that *Streptocarpus* contains chalcones, since Bopp[22] reported that the yellow spot on the flower of ten species of this genus was flavonoid in nature; his data strongly indicate that he was dealing with chalcones. The fact that four of

[20] T. R. SESHADRI, *Rev. Pure Appl. Chem.* **1**, 186 (1951).
[21] J. B. HARBORNE, *Phytochem.* **5**, 111 (1966).
[22] M. BOPP, *Naturwissenschaften* **47**, 159 (1960).

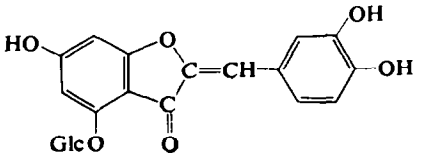
the five genera mentioned above belong to one tribe, the Didymocarpeae, in the Cyrtandroideae is probably only a reflection of the large size of this tribe: wider sampling is clearly needed.



(VII) R = H; Pedicin
(VIII) R = CH_3; Pedicellin



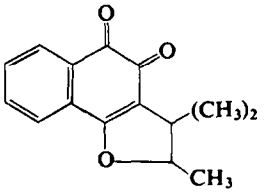
(IX) Chalcononaringenin



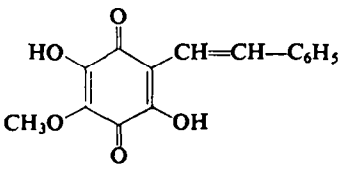
(X) Cernuoside

Flavonols seem to be completely absent from the Gesneriaceae. They were looked for but not found in any of the material under study (46 species). There is an earlier report of kaempferol occurring in garden forms of *Streptocarpus*.[23] This flavonol was found only in flowers of the bottom recessive pink genotype and all other *Streptocarpus* material, both cultivars and species, contained only apigenin or luteolin. This is clearly a special case and flavonol production is not a characteristic feature of the genus. An exactly similar situation exists in *Dahlia variabilis* (Compositae); the flowers of nearly all colour varieties contain apigenin and luteolin glycosides whereas kaempferol and quercetin occur only in some bottom recessive white forms.[24]

Quinone pigments are also known in the Gesneriaceae and appear to be restricted, like the chalcones and aurones, to the Cyrtandroideae; they have been found so far only in *Streptocarpus* and *Didymocarpus*. Dunnione (XI) occurs as an orange-red deposit on the leaves of *S. dunnii*.[25] The presence of this rare α-naphthoquinone on the leaves of a closely related species, *S, pole-evansii*, has now been confirmed by spectral analysis and by thin-layer chromatography in three solvent systems. The quinone in *Didymocarpus pedicellata*



(XI) Dunnione



(X) Pedicinin

is also a leaf deposit and is pedicinin (XII), which is related to the chalcones (VII and VIII) already mentioned. A new unnamed *Didymocarpus* species found by B. L. Burtt (Accession no. C4304) has a brownish-yellow deposit on its leaves and preliminary studies in this

[23] J. B. Harborne, In *Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) p. 602. Pergamon Press, Oxford (1962).
[24] E. C. Bate-Smith, T. Swain and C. G. Nordström, *Nature* **176**, 1016 (1955).
[25] J. R. Price and R. Robinson, *J. Chem. Soc.* 1522 (1939); 1493 (1940).

laboratory indicate that quinones are present here too. None of the many other gesnerads studied during this survey appeared to have quinones.

DISCUSSION

The present discovery of 3-desoxyanthocyanins in eight fern species, the earlier report by Bendz *et al.*[10] of luteolinidin (I) in the moss *Bryum* and the general rarity of this kind of pigment in higher plants suggest that the presence of 3-desoxyanthocyanins is phylogenetically "primitive". Biogenetic considerations also indicate that 3-desoxyanthocyanins are simpler than the 3-hydroxylated anthocyanins, requiring as they do one less oxidation in their synthesis from a chalcone precursor. If this idea is accepted, then the production of 3-desoxyanthocyanins in the highly advanced family, the Gesneriaceae, is anomalous. The other reported occurrences of 3-desoxyanthocyanins in the Bignoniaceae (a family closely related to the Gesneriaceae) and in *Sorghum* (Poaceae) present similar difficulties.

The most likely explanation is that these pigments are synthesized in the Gesneriaceae in response to selection for brilliant scarlet flower colour, which is favoured by bird pollinators. It is significant that 3-desoxyanthocyanins are restricted to New World species, which are mainly tropical plants, since ornithophily (bird pollination) is known to be widespread in the tropical Tubiflorae.[26] *Gesneria* and *Kohleria* plants growing in Jamaica are known to be attended by humming birds or by the bananaquit (*Coereta flaveola*).[27] A parallel situation exists in the Labiatae; the long-tubed red *Salvia splendens* of the Andes is known to be ornithophilous, whereas the temperate blue-flowered *Salvias* are bumble-bee flowers. There is no suggestion of 3-desoxyanthocyanins occurring in red *Salvia*, since acylated pelargonidin 3,5-diglucosides have been isolated from this species.[28] Nevertheless, the 3-desoxyanthocyanins of the Gesnerioideae represent an efficient chemical means of producing the same visual effect.

Alternatively, it is possible that 3-desoxyanthocyanins are formed in higher plants by a different route from that employed in lower plants or that the enzyme system for 3-hydroxylation is lost during evolution. There is in fact, a general trend for flavonols to be replaced by flavones in the leaves of higher plants, since Bate-Smith[2] has indicated that flavonols are eliminated in the changeover from woody to herbaceous habit. There is no obvious reason why the same trend should not affect anthocyanin synthesis. In the case of the Gesneriaceae, it is interesting that flavonols are absent from the leaves and flowers. Loss of an enzyme for 3-hydroxylation might well occur in both organs. Indeed, 3-desoxyanthocyanins occur as extensively in leaves (12 out of 21 species examined) as in flowers (11 out of 21 species) of the Gesnerioideae.

Although discussion of the origin of 3-desoxyanthocyanins in higher plants is still necessarily speculative, their systematic importance in the Gesneriaceae is on firmer ground. The present survey has not been extensive but the distribution pattern observed is highly significant: the presence of 3-desoxyanthocyanins is correlated with isocotyly and geographical restriction to the New World in delineating the sub-family Gesnerioideae. Further, other correlated chemical differences probably also occur: thus chalcones and aurones have only so far been found in the Cyrtandroideae and quinones are known only in two genera (*Streptocarpus* and *Didymocarpus*) of this sub-family; by contrast, methylated flavones seem to be

[26] L. VAN DER PIJL, *Evolution* **15**, 44 (1961).
[27] R. SUTTON, Private communication.
[28] J. B. HARBORNE, *Phytochem.* **3**, 151 (1964).

restricted to the Gesnerioideae. Further surveys will indicate whether these other apparent chemical differences are significant or not.

3-Desoxyanthocyanins have only been found in one other family in the order Tubiflorae, the Bignoniaceae—an isolated occurrence in the genus *Arrabidaea*; they may be present elsewhere in the family, since surveys have been limited so far by the inaccessibility of material (the family is tropical American). There is little doubt, however, that 3-desoxyanthocyanins are absent from the Scrophulariaceae and the Orobanchaceae, two families closely allied to the Gesneriaceae, ones which have been extensively studied. Thus the flavonoid pattern of the Gesneriaceae is distinctive in terms of these rare anthocyanins. The other characteristic flavonoids of the Gesneriaceae (chalcones, aurones and methylated flavones) appear in a number of other families of the Tubiflorae.

EXPERIMENTAL

Plant Material

Most ferns were grown from spores in the glasshouse and were submitted to the Keeper of Ferns, Royal Botanic Gardens, Kew, for identification. Other material was obtained directly from Kew, from the Royal Park, Windsor or from the Botanic Gardens, Glasgow.

Leaves and petals of most of the Gesneriaceae were supplied by B. L. Burtt, Royal Botanic Garden, Edinburgh. These plants bear C. numbers (Table 2), indicating that preserved specimens have been deposited in the Herbarium, Edinburgh. A few Gesneriaceae were also obtained from Kew and the remainder were growing at the John Innes Institute.

Authentic Pigments

Gesnerin and luteolinidin 5-glucoside were isolated in crystalline state from petals of *Rechsteineria cardinalis*. The 3-desoxyanthocyanins were synthesized by condensing *O*-benzoylphloroglucinaldehyde with the appropriate acetophenone.[29] Tricetinidin was obtained by demethylation of tricinidin with pyridinium chloride. 6-Hydroxypelargonidin (cf.[30]) was prepared by condensing 2,5-dihydroxy-4,6-dimethoxybenzaldehyde, obtained by Elbs perulphate oxidation of 2-hydroxy-4,6-dimethoxybenzaldehyde, with ω,4-diacetoxy-acetophenone, followed by demethylation of the product. 6-Hydroxycyanidin was obtained by reductive acylation, followed by acid treatment, of quercetagetin.

Pigment Identification—General

All anthocyanins were identified by direct spectral and chromatographic comparisons with authentic substances, and by analysis of their hydrolysis products. The properties of the new glycosides of apigeninidin and luteolinidin found in ferns are given in Table 2. Solvent system abbreviations in this Table and in Table 1 are as follows: Forestal, acetic acid–conc. HCl–water (30:10:3); Formic, formic acid–conc. HCl–water (9:2:3); BAW, butanol–acetic acid–water (4:1:5); EtAc-HCO_2H, ethyl acetate–formic acid–2NHCl (85:9:6) (this solvent was used for thin-layer chromatography on silica gel G); Bu HCl, butanol–2NHCl (1:1, top layer); HOAc-HCl, acetic acid–conc. HCl–water (15:3:82). Spectra were measured on a Unicam SP 500 spectrophotometer.

Flavones were identified in acid-hydrolysed leaf or petal extracts by chromatography and

[29] A. Leon and R. Robinson, *J. Chem. Soc.* 2734 (1931).
[30] E. H. Charlesworth and R. Robinson, *J. Chem. Soc.* 1619 (1934).

co-chromatography in five solvent systems with authentic compounds. Except for the two common flavones, these identifications were confirmed by spectral measurements.

Identification of Individual Pigments

The diosmetin glycoside in *Columnea* × *banksii* flowers was isolated by paper chromatography in BAW (R_f 0·20), 15% HOAc and water (R_f 0·75) (colour in u.v. light: dull brown, unchanged with NH_3). Its spectral properties were $\lambda_{max.}^{EtOH}$ 257, 271, 348; $\lambda_{max.}^{EtOH\text{-}NaOAc}$ 257, 271; $\lambda_{max.}^{EtOH\text{-}H_3BO_3}$ 257, 270, 348; $\lambda_{max.}^{EtOH\text{-}AlCl_3}$ 352, 375; $\lambda_{max.}^{EtOH\text{-}NaOH}$ 275, 375 mμ. On acid hydrolysis, it gave diosmetin and glucose.

Columnin was isolated from flowers of various *Columnea* species by extraction with hot alcohol, and the concentrated pigments deposited dark-red crystals on standing. The crude pigment was purified by solution in methanol, followed by ether precipitation, and by paper chromatography. The cruder samples had $\lambda_{max.}$ 269, 345, 420 (inflection), 517 mμ, the peak at 345 mμ, indicating the presence of flavone impurity; purer material showed little or no absorption in the 320–380 region. Columnin gave a purple colour in MeOH/$AlCl_3$ and a blue colour in MeOH/NaOH. On acid hydrolysis, columnin gave glucose and columnidin. The material so obtained still retained some impurity but on treatment with pyridinium chloride at 100° for 1 hr gave pure anthocyanidin (Table 1). The molecular weight, determined by mass spectroscopy, was 287. Alkaline cleavage of columnin was carried out with Na/Hg in 1 N NaOH under N_2 at 100° for ½ hr and the products were identified by two-dimensional thin-layer chromatography.[14]

Cernuoside (aureusidin 4-glucoside) was isolated from flowers of *Petrocosmea kerrii* by extraction with hot EtOH, followed by paper chromatography. The pigment had $\lambda_{max.}^{EtOH}$ 405 mμ, did not separate from added cernuoside in five solvent systems and gave aureusidin on hydrolysis.

Monardein was isolated from fronds of *Davallia divaricata* by extraction with 1% MeOH-HCl and purified by paper chromatography. The main pigment was identified as monardein on the following evidence: (1) it had $\lambda_{max.}$ 278, 313, and 508 mμ., with $E_{313}E_{505}=63\%$ and $E_{440}/E_{508}=19\%$; (2) it did not separate from added monardein in four solvent systems; and (3) it gave pelargonin and *p*-coumaric acid on alkaline hydrolysis; and (4) it gave pelargonidin on acid hydrolysis. Three minor pigments were also present. Two were identified as pelargonin and cyanin, the third, from its R_fs and colour properties, appeared to be an acylated cyanidin glycoside.

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