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COMPARATIVE BIOCHEMISTRY OF THE FLAVONOIDS—VI.

FLAVONOID PATTERNS IN THE BIGNONIACEAE AND THE GESNERIACEAE

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Abstract—3-Desoxyanthocyanins which characterize the sub-family Gesnerioideae of the Gesneriaceae have been reported once in the closely related Bignoniaceae, in *Arrabidaea*. A search of sixteen representative species has now failed to reveal any further occurrences of these rare plant pigments in the Bignoniaceae. The common anthocyanin, cyanidin 3-rutinoside was found, instead, in *Campsis radicans*, *Catalpa bignonioides*, *Eccremocarpus scaber*, *Incarvillea mairei* and *Tecoma garrocha* petals. Orange and yellow petal colours in *Anemopaegma chamberlaynii* and *Pyrostegia venusta* were found to be due to carotenoids, not to 3-desoxyanthocyanins. An examination of the other flavonoids in leaf and petal of bignoniads showed that most species contained flavones rather than flavonols. A new pigment, 5,6,7,3′,4′-pentahydroxyflavone (6-hydroxyluteolin) was identified in fresh leaves of *Catalpa bignonioides* and *C. speciosa* and in herbarium material of *C. bungei* and *Tecoma australis*. An earlier chemotaxonomic survey of flavonoids in leaf and flower of the closely allied Gesneriaceae has been extended to a further twenty-five species and the previously noted differences in flavonoid pattern at the sub-family level have been confirmed. 3-Desoxyanthocyanins were identified in a further eleven species of the Gesnerioideae and the yellow chalcone isosalipurposide or the yellow aurone cernuoside noted in a further six species of the Cyrtandroideae. Preliminary evidence was obtained that a *C*-glycosylcyanidin derivative occurs in sepals of *Cyrtandra pendula*.

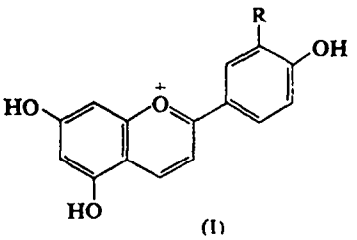
INTRODUCTION

THE Tubiflorae, as defined in the latest edition[1] of Engler's Syllabus, is one of the largest of all plant orders including as it does some twenty-six phyletically advanced families. Certain families are so closely similar to each other morphologically that classification is difficult and some taxonomists prefer to regard the order as a single super-family, containing a large number of tribes within its bounds. This is the kind of situation in which chemical data might be of assistance in classification and already, Darnley-Gibbs,[2] using simple colour tests, has carried out a brief chemical survey of the group. Since families comprising the order are known to be rich in unusual flavonoids (for refs., see Ref. 3), surveys of the various families are being carried out in order to extend the chemical approach.

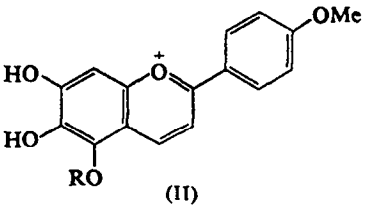
In an earlier paper on the Gesneriaceae flavonoids,[4] it was reported that the rare 3-desoxyanthocyanidins apigeninidin (I, R = H), luteolinidin (I, R = OH) and columnidin (6- or 8-hydroxyluteolinidin) occur as regular constituents in leaf and petal of members of the New World sub-family, the Gesnerioideae. Following this discovery, it was of interest to see whether this unusual class of pigment, responsible for the orange–red petal colours favoured by bird pollinators, occurred at all regularly in any other Tubiflorae family. The

[1] A. ENGLER, *Syllabus der Pflanzenfamilien*, 12th edn (edited by H. MELCHIOR), Vol. II. Borntraeger, Berlin (1964).
[2] G. DARNLEY-GIBBS, *Trans. Roy. Soc. Can.* Ser. 3 **56**, 161 (1962).
[3] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*. Academic Press, New York (1967).
[4] J. B. HARBORNE, *Phytochem.* **5**, 589 (1966).

Bignoniaceae are of especial interest in this respect since they are mainly New World plants known to be widely pollinated by birds. In addition, related pigments carajurin (II, R = Me)* and carajurone (II, R = H)* have been reported once, in the leaf of *Arrabidaea chica* (formerly *Bignonia chica*).[5]



(I)



(II)

A survey has therefore been carried out of all available members of the Bignoniaceae, especially of species with yellow, orange or orange–red flower colour. At the same time, the other flavonoids were examined to see whether the leaves predominantly contained flavones (e.g. luteolin) or flavonols (e.g. quercetin). This is a point of some phylogenetic interest, since it has earlier been shown that the ratio of species with flavones to those with flavonols is an indication of evolutionary advancement among families of the Tubiflorae.[6] The opportunity has been taken here to report a further survey of members of the Gesneriaceae, which confirms and extends the earlier chemotaxonomic findings in this family.[4] The general bearing of the flavonoid results reported in this paper on the systematics of the Tubiflorae has already been considered briefly elsewhere.[3]

RESULTS

Flavonoids of the Bignoniaceae

The Bignoniaceae, a family of some 100 genera and 650 species, are mainly climbing plants growing in the forest vegetation of tropical South America, and fresh plant material for phytochemical study is generally inaccessible. The present survey has perforce been limited largely to the handful of species grown in this country as ornamental plants, e.g. the tree *Catalpa bignonioides*. The survey has, however, included petal material of a West Indian species *Pyrostegia venusta* (formerly *Bignonia venusta*), a plant which is probably bird-pollinated and which has a flower colour (orange) which could be based on 3-desoxyanthocyanin. The survey was extended by using dried leaf from herbarium specimens of six further species; analyses of floral tissue taken from herbarium sheets were unfortunately not satisfactory because petal pigments are usually destroyed during the drying process.

The combined results of surveying these sixteen species for flavonoids are presented in Table 1. The very common anthocyanin, cyanidin 3-rutinoside was identified in five species. This is a pigment new to the Bignoniaceae; the only other anthocyanins that have been fully identified in the family are the 3-glucoside and 3,5-diglucoside of delphinidin, in petals of *Jacaranda acutifolia*.[7] Three other species with orange and yellow petals were found (Table 1)

* Pigments are written in the flavylium form to emphasize their relationship with other desoxyanthocyanidins; they are more usually given in the quinone form.

[5] E. CHAPMAN, A. G. PERKIN and R. ROBINSON, *J. Chem. Soc.* 3015 (1927).

[6] J. B. HARBORNE, In *Comparative Phytochemistry* (edited by T. SWAIN), pp. 271–295. Academic Press, New York (1966).

[7] J. BILLOT, *Compt. Rend.* **258**, 2386 (1964).

to have carotenoids, not anthocyanins. Thus, the present results, combined with those of an earlier survey[8] in which pelargonidin, cyanidin or delphinidin glycosides were detected in seven species from seven genera, indicate that 3-desoxyanthocyanins such as apigeninidin or luteolinidin are apparently absent from the family. The cosmetic pigments carajurin and carajurone of *Arrabidaea chica* leaf, with their unusual structures based on 5,6,7,4′-tetrahydroxyflavylium, remain the only known 3-desoxyanthocyanins in the Bignoniaceae. It may be noted in passing that 3-desoxyanthocyanins have not been found in any other Tubiflorae family as yet. An earlier suggestion[9] that such pigments occur in petals of *Holmskioldia sanguinea* (Verbenaceae) has not been confirmed by a detailed analysis; the anthocyanin has been identified as the acylated pelargonidin glycoside monardein and there is no trace of other pigments.

TABLE 1. FLAVONOID DISTRIBUTION IN THE BIGNONIACEAE

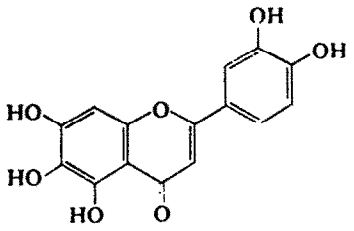
Tribe, genus and species	Source*	Petal flavonoids	Leaf flavonoid aglycones
Bignonieae			
Anemopaegma chamberlaynii	BG	None (carotenoids)	None
Cydista aequinoctialis	LH	—	Quercetin
Pyrostegia venusta	WI	None (carotenoids)	—
Clytostoma callistegioides	BG	None (carotenoids)	Methylated flavone
Tecomeae			
Campsis radicans Seem.	BG	Cyanidin 3-rutinoside	Luteolin (as 7-glucoside)
Catalpa bignonioides Walt.	BG	Cyanidin 3-rutinoside	Luteolin (as 7-glucoside) and 6-hydroxyluteolin
C. bungei Mey	LH	—	Luteolin and 6-hydroxyluteolin
C. speciosa Ward	BG	—	Luteolin and 6-hydroxyluteolin
Chilopsis saligna Don	LH	—	Luteolin and apigenin
Incarvillea mairei	BG	Cyanidin 3-rutinoside	Quercetin, kaempferol and flavones
Podranea ricasoliana	BG	—	Flavones
Tecoma australis R. Br.	LH	—	Luteolin and 6-hydroxyluteolin
T. garrocha Hieron.	BG	Cyanidin 3-rutinoside	Luteolin
Tecoma species	LH	—	Flavones
T. stans Juss.	LH	—	Quercetin and kaempferol
Eccremocarpeae			
Eccremocarpus scaber Ruiz & Pav	BG	Cyanidin 3-rutinoside	None

* BG = botanic garden (fresh material); LH = University of Liverpool herbarium (dried material); WI = West Indies (supplied by Dr. Adams, Jamaica).

A study of leaf and petal flavonoids in the family indicates that luteolin is a common constituent, being present in at least seven species. This flavone, provisionally noted by Bate-Smith[10] in hydrolysed leaf extracts of *Campsis radicans*, was fully identified in this plant and the glycoside in unhydrolysed extracts determined as luteolin 7-glucoside. The same glucoside also occurs in the leaf of *Catalpa bignonioides*, from which Birkofer and his coworkers[11] isolated it as a *p*-coumaryl ester very recently. These authors also overlooked as

[8] W. G. C. FORSYTH and N. W. SIMMONDS, *Proc. Roy. Soc.* **B142**, 549 (1954).
[9] J. B. HARBORNE, In *Chemical Plant Taxonomy* (edited by T. SWAIN), pp. 359–388. Academic Press, New York (1963).
[10] E. C. BATE-SMITH, *J. Linn. Soc. (Botany)* **58**, 39 (1962).
[11] L. BIRKOFER, C. KAISER and H. KOSMOL, *Z. Naturforsch.* **20b**, 605, 923 (1965).

a major glycoside of the same plant a new flavone, here identified as 5,6,7,3',4'-pentahydroxyflavone (III) or 6-hydroxyluteolin. Identification of the aglycone as a 6-substituted flavone was based, in the first instance, on the characteristic dark absorbing colour on paper in u.v. light and the unusual short u.v. band in the spectrum at 285 nm, instead of at 275 nm. The R_f data when compared with known flavones indicated that it should be the 6-hydroxy derivative of luteolin and this was confirmed by analysis and by comparison with synthetic material obtained by demethylation of the pentamethyl ether, sinensetin.[12]



(III) 6-Hydroxyluteolin

6-Hydroxyluteolin is also present in *C. speciosa* leaf and a search of the herbarium showed it to be present in *C. bungei* and in *Tecoma australis*. Leaf material from a specimen of the latter plant collected in 1881 was used for this analysis and it is noteworthy that a flavone with the unstable pyrogallol nucleus should be retained unchanged for so many years. 6-Hydroxylation is clearly a common structural feature of the Bignoniaceae flavonoids, for, besides the luteolin derivative now found in *Bignonia* and *Tecoma*, the simpler 5,6,7-trihydroxyflavone and its 6-methyl ether have been known for some time as bark and seed constituents of *Oroxylum indicum*.[13] 6-Hydroxylation is, of course, also part of the structures of the red pigments carajurin (II, R = Me) and carajurone (II, R = H) of *Arrabidaea*. It is of systematic interest that 6-hydroxyflavones are also known in the Labiatae, Scrophulariaceae and Verbenaceae (cf. Ref. 3).

Several unidentified flavones were also detected during this survey. One found in leaf of *Clytostoma callistegioides* appears to be an *O*-methylated flavone (high R_f in Forestal, dark-brown colour in u.v. light unchanged by ammonia). Although similar in spectrum to acacetin (see Experimental) it was chromatographically different and could not be equated with any known flavone. Flavonols were found in only three species and of the sixteen species surveyed, no less than eleven contained flavones. This result links the Bignoniaceae with families such as the Acanthaceae and Gesneriaceae, which also have flavones as regular leaf or petal constituents and separates it clearly from predominantly flavonol-containing families, such as the Solanaceae and Convolvulaceae (see Refs. 3 and 6).

Flavonoids of the Gesneriaceae; a Further Survey

In an earlier survey of forty-six gesnerads drawn from nineteen genera, the distribution of flavonoids was shown to be correlated with systematics at the sub-family level.[4] Thus, 3-desoxyanthocyanins were found in eighteen of the twenty-one species of the isocotylous New World Gesnerioideae, but were reported absent from twenty-five species of the anisocotylous Old World Cyrtandroideae. A further twenty-seven species have now been examined

[12] L. J. SWIFT, *J. Org. Chem.* **30**, 2079 (1965).
[13] P. K. BOSE and S. N. BHATTACHARYYA, *J. Indian Chem. Soc.* **15**, 311 (1938).

(Tables 2 and 3) and desoxyanthocyanins have been isolated from eleven of fifteen Gesnerioideae, but could not be found in twelve more Cyrtandroideae. These results bring the total figures for 3-desoxyanthocyanins in the Gesnerioideae of twenty-nine in thirty-six species, for their absence from the Cyrtandroideae to thirty-seven species. At the generic level, the

TABLE 2. A FURTHER SURVEY OF THE FLAVONOIDS IN THE GESNERIACEAE: PIGMENTS OF THE SUB-FAMILY GESNERIOIDEAE

Tribe, genus and species	Source‡	Anthocyanins	Other pigments†
New World Species (sub-family Gesnerioideae)			
Columneae			
**Chrysothemis pulchella* Decne.	Cald.	Luteolinidin 5-glucoside and gesnerin (sepals) and cyanidin 3-rutinoside (petals)	Luteolin
**Columnea crassifolia* Hook.	Kew	Columnin (petal and/or leaf)	Diosmetin and/or luteolin, petal carotenes (see text)
**C. linearis* Oerst.	Cald.		
**C. rutilans* Sw.	Kew		
**C. woodii*	Kew		
**Episcia cupretta* cv. "Metallica" (Hook) Hanst.	Cald.	Pelargonidin 3-rutinoside (petal) and columnin (leaf)	Luteolin
**E. melittifolia* Mart.	Cald.	Cyanidin 3-rutinoside (petal), luteolinidin glycoside and columnin (stem)	None
**Hypocyrta glabra* Hook.	Cald.	Luteolinidin 5-glucoside (petal)	Luteolin
Coronantheae			
Rhabdothamnus solandri A. Cumm.	C. 2930	Cyanidin and pelargonidin 3-rutinosides (petal)	Luteolin
Gesnerieae			
Rhytidophyllum tomentosum Mart.	Cald.	Delphinidin and cyanidin glycosides (petal)	None
Kohlerieae			
**Kohleria bogotensis* Fritsch.	Cald.	Pelargonidin 3-rutinoside (petal) and columnin (leaf)	None
Mitrarieae			
Fieldia australis A. Gunn.	C. 4625	Absent	Luteolin and unidentified flavonol(s)
**Sarmienta repens* Ruiz & Pav	C. 5165	Luteolinidin glycoside (petal)	Luteolin
Sinningieae			
**Rechsteineria macrorhiza*	Cald.	Luteolinidin 5-glucoside and gesnerin (petal)	Luteolin
Tribe?			
Titanotrichum oldhamii (Hemsl.) Solereder	C. 2900	Cyanidin 3-rutinoside (stem and petal)	Luteolin and carotenes (in petal)

* Species with 3-desoxyanthocyanins.

† Flavones occur in petal and/or leaf as 7-glycosides.

‡ Cald. denotes plants which were obtained from Calderstones Park, Liverpool. All other plants were obtained from Edinburgh Botanic Garden and the C. number is Burtt's reference number; herbarium specimens are available.

two surveys have covered thirty-two genera, nearly 40 per cent of those described. The additional data thus confirm strongly the correlation between chemistry and systematics in the family and indicate that the 3-desoxyanthocyanin is useful as a sub-family character.

The fact that 3-desoxyanthocyanins could not be detected in seven of the Gesnerioideae species examined may not be a serious handicap to their use in systematics. In five of the species, lack of a positive test was either due to the fact that both leaf and flower tissue of the species in question were not available for analysis or because leaf or flower lacked cyanic

TABLE 3. A FURTHER SURVEY OF FLAVONOIDS IN THE GESNERIACEAE: PIGMENTS OF THE SUB-FAMILY CYRTANDROIDEAE

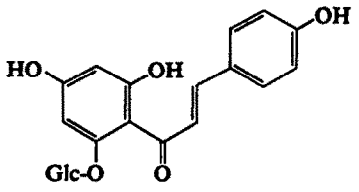
Tribe, genus and species	Source*	Anthocyanins	Other pigments
Old World Species (sub-family Cyrtandroideae)			
Cyrtandreae			
Cyrtandra oblongifolia (Bl.) C.B. Cl.	C.5171	None	Cernuoside (petal) and quinone (leaf)
C. pendula Bl.	C. 4038	*C*-Glycosylcyanidin (?) glycosides (sepal)	Isosalipurposide (petal)
Rhynchotechum discolor (Maxim.) B. L. Burtt	C. 3711	None	Luteolin
R. parviflorum (Blume)	C. 5121	Petunidin 3-sophoroside	None
Didymocarpeae			
Didymocarpus corchorifolius Wall.	C. 5168	Cyanidin 3-glucoside (stem)	Quinone (?) (leaf)
D. humboldtianus Gardn.	Cald.	Delphinidin glycoside (petal)	Unidentified aurone (petal)
D. malayanus	C. 5133	None	Cernuoside (petal) and quinone (?) (leaf)
Jerdonia indica Wight	C. 5117	Cyanidin 3-sambubioside (petal)	Luteolin
Trichosporeae			
Aeschynanthus ellipticus Diels.	Cald.	Cyanidin 3-sambubioside (petal)	Isosalipurposide (petal)
A. marmoratus T. Moore		Cyanidin 3-sambubioside (petal)	None
A. tricolor Hook f.	C. 4042	Cyanidin and pelargonidin 3-sambubiosides (petal)	Isosalipurposide (petal)
Agalmyla parasitica (Lam.) Kuntze	C. 2854	Pelargonidin 3-sambubioside-5-glucoside	Quinone (?) (leaf)

* See Table 2.

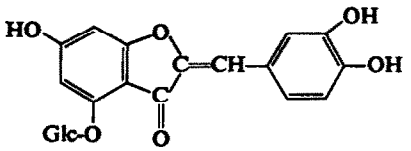
colour. The latter difficulty might be circumvented by inducing pigment synthesis in otherwise green leaves by altering the environment and there is good evidence that gesnerads would respond in this way to low temperatures or increased light intensities. Both leaf and petal of the other two species, *Titanitrichum* and *Rhabdothamnus*, were available for study and there seems to be little doubt that both lack desoxyanthocyanins but it is significant that these species happen to be taxonomically anomalous, i.e. ones which do not fit particularly well on morphological grounds into either sub-family[14] (see also below).

[14] B. L. BURTT, *Notes Roy. Botan. Garden Edinb.* **24**, 205 (1962); and unpublished observations.

In the earlier survey,[4] chalcones and aurones were reported in six genera of the Cyrtandroideae and the suggestion was then made that yellow flavonoid pigments were characteristic of this sub-family just as the 3-desoxyanthocyanins were characteristic of the Gesnerioideae. This has now been confirmed by the discovery of isosalipurposide (IV) in petals of two further *Aeschynanthus* species (Table 3) and in *Cyrtandra pendula*, of cernuoside (V) in *C. oblongifolia* and in *Didymocarpus malayanus* and of an unidentified aurone in *D. humboldtianus*. The correlation with systematics is good in that chalcones and aurones have now been found in a third of the Cyrtandroideae taxa examined (i.e. twelve out of thirty-seven species) but have not been detected in a single Gesnerioideae (thirty-six species examined). What is more, there is now evidence that these water-soluble flavonoids (IV) and (V) are replaced as yellow pigments by lipid-soluble carotenoids in the sub-family Gesnerioideae. Thus, carotenoids have been detected in many *Columnea* species, e.g. in the bright yellow corollas of *C. kucyniakii* and *C. tulae* var. *flava* and (mixed with the anthocyanidin columnidin) in the orange–red corollas of *C.* × *banksii*, in *Alloplectus ambiguus* and in *Titanotrichum oldhamii* (all Gesnerioideae).



(IV) Isosalipurposide



(V) Cernuoside

Evidence is accumulating during these surveys that the glycosidic patterns of the anthocyanins may be related to systematics, especially at the generic or tribal level. For example, the common 3-rutinoside (3-rhamnosylglucoside) is replaced by 3-sambubioside (3-xylosylglucoside) in *Aeschynanthus*; pelargonidin and/or cyanidin 3-sambubioside were found in both *Aeschynanthus* species examined previously[4] and have now been found in three further species. Other occurrences of 3-sambubiosides or related types are in *Agalmyla* (same tribe) and in *Jerdonia* (same sub-family). Another glycosidic type different from the 3-rutinoside has now been found in *Rhyncotechum parviflora* and a pigment which appears from R_f data to be the so far undescribed 3-sophoroside of petunidin is present in the petals of this plant. The most interesting glycoside found during the present survey occurs in the sepals of *Cyrtandra oblongifolia*. This is a cyanidin derivative with unusual chromatographic properties; it has a rather low R_f in butanolic solvents but an exceptionally high mobility in aqueous solvents (R_f 0·74 in 1% aqu. HCl). On prolonged acid treatment, it loses some sugar but yields a pigment which is still mobile in water, indicating that a *C*-glycosyl sugar is present in the original pigment. Lack of plant material has prevented further study but there is a strong suggestion that a pigment with both *O*- and *C*-glycosyl attachments occurs in this plant. *C*-Glycosyl derivatives of most flavonoid classes are now known,[3] but no *C*-glycosylanthocyanin has yet been reported.

From the earlier survey,[4] it appears that flavonols were absent from the Gesneriaceae and this has generally been confirmed by the present studies in which the flavones luteolin or diosmetin have been detected in most species. Three compounds behaving like flavonols were, however, noted exceptionally in the cream petals of *Fieldia australis*, a New South

Wales plant which is included in the Gesnerioideae because it is isocotylous. The three compounds in the petals differed in R_f from any of the common flavonols, but had the typical bright yellow colour in u.v. light on paper of flavonols and one had u.v. spectral maxima at 259 and 378 nm, indicating that it was a flavonol not a flavone. The leaves of *Fieldia* contain a more usual flavonoid constituent, luteolin 7-glucoside. Several other unidentified yellow pigments were noted in the Cyrtandroideae. From their general behaviour (they have high R_f values in Forestal, dull u.v.-absorbing colours on paper and atypical u.v. spectra), they appear to be related to the chalcone quinones, pedicellin and pedicin, isolated earlier from the orange deposits on leaves of *Didymocarpus pedicellata*.[15] This supposition is strengthened by the fact that they were present in two of the three *Didymocarpus* species studied and one of the two, *D. corchorifolius*, had pigment granules on the leaf surface. These quinone-like substances were also noted in *Agalmyla* and *Cyrtandra*, and are thus, like chalcones and aurones, confined to the Cyrtandroideae.

Four of the twenty-seven new Gesneriaceae species studied—*Fieldia*, *Jerdonia*, *Rhabdothamnus* and *Titanotrichum*—are taxonomically "difficult", i.e. they are difficult to place with certainty in either of the two sub-families. Taxonomic studies of *Jerdonia* are still in progress and it is not clear whether it has been placed in the right family (it could be a member of the Scrophulariaceae).[14] The chemical data regrettably do not help much in placing these anomalous taxa. Thus, *Titanotrichum* has yellow carotenoid petal pigments characteristic of the Gesnerioideae, but lacks 3-desoxyanthocyanin, the most taxonomically significant pigment in the sub-family. Similarly, *Jerdonia* contains cyanidin 3-sambubioside found also in *Aeschynanthus*, which places it in the Cyrtandroideae, but this glycosidic type is also known in the Scrophulariaceae (in *Nemesia strumosa*).[3] One can only conclude, as Burtt[14] has done, that difficult plants, both chemically and morphologically, tend to take up a neutral position with regard to what is otherwise a clear-cut natural division of the family.

EXPERIMENTAL

Plant Material

Fresh leaves and flowers of Bignoniaceae were obtained from plants growing at Ness Gardens, Neston, Cheshire, or Calderstones Park, Liverpool. Dried leaves of Bignoniaceae were obtained from the University of Liverpool herbarium by kind permission of Professor V. H. Heywood. Fresh petals of *Pyrostegia venusta* were sent by airmail from the West Indies by Dr. C. D. Adams, Jamaica, and of *Holmskiolda sanguinea* by Dr. C. E. Seaforth, Trinidad. Leaves and petals of most Gesneriaceae were supplied by B. L. Burtt, Royal Botanic Gardens, Edinburgh. These plants bear C numbers, indicating that preserved specimens have been deposited in the herbarium, Edinburgh. Leaves and petals of the remaining Gesneriaceae were obtained from Calderstones Park; most of these plants were raised from seed supplied by Edinburgh and their identity is reasonably secure.

Pigment Identifications

These were carried out as described in earlier papers in this series e.g.[4] Chromatography solvent abbreviations are as used in earlier papers. Spectra were measured on a Unicam SP 800 Spectrophotometer.

6-Hydroxyluteolin

This pigment was isolated from leaf of *Catalpa bignonioides* as the 7-glucoside along with luteolin 7-glucoside. The 7-glucoside had λ_{max}^{EtOH} 255, 285 and 345 nm, $\Delta\lambda^{AlCl_3}$ +30 nm, $\Delta\lambda^{H_3BO_3}$ +20 nm and $\Delta\lambda^{NaOEt}$ + 55 nm. R_f values were (values for luteolin 7-glucoside in parenthesis): 0·24 (0·45) in BAW, 0·01 (0·03) in H_2O, 0·08 (0·15) in 15% HOAc and 0·46 (0·67) in PhOH. On hydrolysis, the glycoside gave glucose and 5,6,7,3′,4′-pentahydroxyflavone (Found: C, 56·3; H, 3·9. $C_{15}H_{10}O_7.H_2O$ requires: C, 56·2; H, 3·8%). The aglycone appeared on chromatograms as an intense black spot in u.v. light, changing to dark brown with NH_3. It had λ_{max}^{EtOH} 285 and 349 nm, $\Delta\lambda^{AlCl_3}$ +26 nm, $\Delta\lambda^{H_3BO_3}$ +21 nm and $\Delta\lambda^{NaOEt}$ +55 nm. R_f values were

[15] T. R. SESHADRI, *Rev. Pure Appl. Chem.* **1**, 186 (1951).

(values for luteolin in parenthesis) 0·54 (0·78) in BAW, 0·53 (0·66) in Forestal and 0·42 (0·66) in PhOH. The aglycone was identical spectrally and chromatographically with a sample of 6-hydroxyluteolin prepared by demethylation of the pentamethyl ether, sinensetin (kindly supplied by Dr. L. J. Swift, USDA, Winter Haven, Florida). On reductive cleavage, the *Catalpa* aglycone gave phloroglucinol and 3,4-dihydroxyphenylpropionic acid.[16]

Methylated Flavone in Clytostoma

A flavone was isolated from hydrolysed leaf extracts of *C. callestigioides* which had the same colour reactions as acacetin (apigenin 4′-methyl ether). Its spectral properties were as follows: λ_{max}^{EtOH} 273 and 336 nm, $\Delta\lambda^{NaOEt}$ +60 nm, $\Delta\lambda^{AlCl_3}$ +20 nm, $\Delta\lambda^{NaOAc}$ +2 nm and $\Delta\lambda^{H_3BO_3}$ 0 nm. It however separated from acacetin when chromatographed in Forestal (R_f 0·91, acacetin 0·97) but had the same R_f in BAW (0·89) and PhOH (0·95).

C-*glycocyanidin* (?) *Glycosides in* Cyrtandra

Two pigments, with colour reactions of cyanidin glycosides, were isolated from sepals and leaves of *C. pendula*. They had $\lambda_{max}^{MeOH-HCl}$ at 530 nm and R_f values 0·09 and 0·23 in BAW, and 0·74 and 0·44 in 1% HCl. They were combined and hydrolysed for 3 hr under N_2 in 2 N HCl. The pigment extracted into BuOH had $\lambda_{max}^{MeOH-HCl}$ at 545 nm and the colour of cyanidin. R_f values of the "aglycone" (cyanidin in parentheses) were 0·61 (0·49) in Forestal and 0·32 (0·22) in Formic. The pigment was mobile in 1% HCl and had an R_f (0·09) between cyanidin (0·02) and cyanidin 3-glucoside (0·12).

Leaf Quinones in Didymocarpus

Several plants of the Cyrtandroideae (see Table 3) contained compounds in the leaf which had high R_f values in most solvents and appeared on paper as yellow in visible light and as dark absorbent spots unchanged by NH_3 in u.v. light. The substance in *D. corchorifolius* leaf had R_f 0·95 in BAW and 0·04 in 5% HOAc and a chalcone-type spectrum with max. at 212, 230 and 340 nm (λ_{max} in EtOH–NaOEt at 290 with inflection at 370 nm). The substance in *D. malayanus* had R_f 0·90 in BAW and 0·51 in 5% HOAc and had a flavanone-type spectrum with max. at 280 and 332 nm (λ_{max} in EtOH–NaOEt at 222, 300 and 384 nm).

Carotenoids in the Bignoniaceae and Gesneriaceae

Yellow and orange pigments were considered to be carotenoids on the basis of (1) solubility in petroleum ether, (2) mobility in thin layers of silica gel in "carotenoid" solvents (e.g. 11% MeOH in benzene), (3) the instability of the colours to light and to oxidizing agents, and (4) the characteristic triplet of spectral peaks in the 400–500 nm region. None was fully identified but most species showed at least two components, one with an R_f close to β-carotene and a second close to lutein. The carotenoid in *Titanotrichum*, after purification, had λ_{max} at 422, 447 and 475 nm in EtOH and could be lutein (which has peaks at 420, 446·5 and 476 in the same solvent). The purified major component in *Pyrostegia* had λ_{max} at 400, 422 and (450) nm in hexane. It differed chromatographically from crocetin (400, 420 and 445 nm) but could be β-zeacarotene (406, 428 and 454 nm). The purified major component of *Columnea kucyniakii* had a rather similar spectrum (λ_{max} 404, 422 and 443 nm in petroleum ether).

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[16] H. M. Hurst and J. B. Harborne, *Phytochem.* 6, 1111 (1967).