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6-HYDROXYLUTEOLIN AND SCUTELLAREIN AS PHYLETIC MARKERS IN HIGHER PLANTS*

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Abstract—A survey of some twelve highly specialized herbaceous families has shown that 6-hydroxyluteolin is present in the majority as a leaf constituent. It has been identified in the Plantaginaceae (*Plantago*), Globulariaceae (*Globularia* and *Lytanthus*), Labiatae (*Amethystea*, *Hemigenia*, *Isanthus*, *Trichostema* and *Westringia*), Buddleiaceae (*Buddleia*, *Chilianthus*) and the Valerianaceae (*Valerianella*). It is accompanied most frequently by luteolin, occasionally by scutellarein and by methylated derivatives. In *Globularia*, 6-hydroxyluteolin was found in leaves of all of the eleven species surveyed; in *G. cordifolia* it was accompanied by its 6-methyl and 6,4′-dimethyl ethers, and by scutellarein and its 6,4′-dimethyl ether (pectolinarigenin). Pectolinarigenin was also identified in *Kerria* (Rosaceae). These distribution patterns are very different from those of 8-hydroxylated flavones and flavonols and indicate that the ability to hydroxylate flavones in the 6-position arose relatively late in evolutionary time.

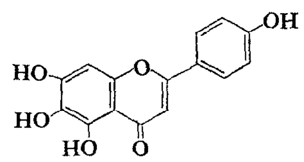
INTRODUCTION

SURVEYS of angiosperm leaf phenolics have established a correlation between flavonoid pattern and evolutionary advancement in that while woody plant families contain predominantly leucoanthocyanidins and flavonols, these compounds (especially leucodelphinidin and myricetin) are generally rare in or absent from herbaceous plant groups.[1,2] It has further been suggested[3,4] that in herbaceous plants, flavones appear to replace flavonols, i.e. that the ability to oxidize the 3-position in the flavanone precursor has been lost during evolution. Examination of a few such families, e.g. the Umbelliferae,[5] Bignoniaceae,[6] Gesneriaceae[6] and Acanthaceae[7] has shown that flavones, such as luteolin, are indeed frequently present. However, a wider survey of herbaceous families such as those in Engler's order Tubiflorae is still needed to confirm this hypothesis. Such surveys are additionally important since a number of the less common flavonoids, i.e. those with extra substituents and potentially of much value as taxonomic markers, are known to occur in isolated instances in some of these families. One such class are the 6-hydroxylated flavones and their derivatives and special attention has now been given to the distribution of these compounds in the Tubiflorae and related groups.

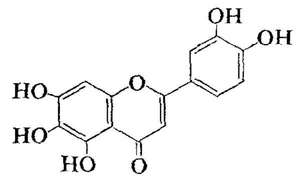
Although scutellarein or 6-hydroxyapigenin (I) was first isolated from *Scutellaria altissima* (Labiatae) as long ago as 1901,[8] the corresponding luteolin derivative (II) was

* Part XIII in the series "Comparative Biochemistry of Flavonoids" for Part XII, see Ref. 23.

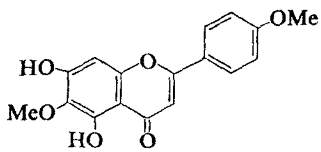
1 E. C. BATE-SMITH, *J. Linn. Soc.* **58**, 95 (1962).
2 E. C. BATE-SMITH, *J. Linn. Soc.* **60**, 325 (1968).
3 J. B. HARBORNE, in *Comparative Phytochemistry* (edited by T. SWAIN), Academic Press, London (1966).
4 J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).
5 R. K. CROWDEN, J. B. HARBORNE and V. H. HEYWOOD, *Phytochem.* **8**, 1963 (1969).
6 J. B. HARBORNE, *Phytochem.* **6**, 1643 (1967).
7 A. G. R. NAIR, S. NAGARAJAN and S. S. SUBRAMANIAN, *Current Sci. India* **34**, 79 (1968).
8 H. MOLISCH and G. GOLDSCHMIDT, *Monatshblat. Chem.* **22**, 679 (1901).



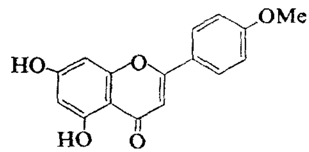
Scutellarein (I)



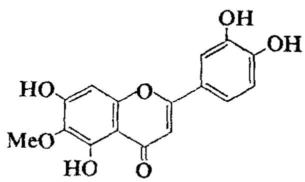
6-Hydroxyluteolin (II)



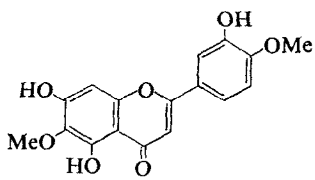
Pectolinarigenin (III)



Acacetin (IV)



Nepetin (V)



(VI)

only discovered recently, in 1967[6] as occurring in *Catalpa* and *Tecoma* (Bignoniaceae). However, methylated derivatives of both (I) and (II) have been described in isolated cases in a number of plant families and the possibility that 6-hydroxyluteolin might be of relatively frequent occurrence in the Tubiflorae has now been confirmed by the present surveys.

RESULTS

As part of a wider chemotaxonomic survey of the Tubiflorae, flavonoids were screened for in direct and acid hydrolyzed leaf extracts of representative samples of a number of Tubiflorae and related families using standard procedures. 6-Hydroxyluteolin, when present, was readily distinguished on chromatograms of acid hydrolyzed extracts by its dark absorbance in u.v. light, unaffected by ammonia fuming, and by its R_f values; it is clearly separated from other flavonoids and phenolic constituents. It was further identified by co-chromatography and by its distinct u.v. spectral properties. Sufficient material was obtained from *Globularia* for an exact mass spectral measurement of its molecular weight (see Experimental).

A number of species showed the presence of *O*-methylated flavones, substances tentatively identified as such by their high R_f values in most solvents and by their dark brown absorbance in u.v. light. Such substances were sometimes obscured by hydroxycinnamic acid derivatives and their identity in *Globularia* only established by more detailed study (see below). Such *O*-methylated flavones may be either derivatives of 6-hydroxyluteolin and scutellarein, e.g. pectolinarigenin (III), or of the common flavones apigenin and luteolin, e.g. acacetin (IV); present chromatographic screening methods are not sufficiently precise to distinguish between these two possibilities.

Flavones of the Globulariaceae

The Globulariaceae is a small herbaceous Tubiflorae family with three genera and 20 species. Apart from two early reports[9,10] of rutin in the leaves of *Globularia alypum* which we have been unable to confirm, nothing is known of the flavonoids in the family. A survey of 12 species from two genera (Table 1) showed that 6-hydroxyluteolin is characteristic and,

TABLE 1. FLAVONE SURVEY OF THE GLOBULARIACEAE

Plant species	Flavone aglycones in leaves		
	6-Hydroxyluteolin	Luteolin	Scutellarein
G. incanescens Viv.*	+	+	–
G. nana Lam.	+	–	–
G. nudicaulis L.	+	+	–
G. spinosa L.	+	–	+
G. vulgaris L.	+	–	–
G. orientalis L.	+	+	+
G. trichosantha Fisch. & Mey.	+	–	–
G. salicina Lam.	+	–	+
G. alypum L.	+	–	–
G. aphyllanthes Crantz	+	–	–
G. cordifolia L.†	+	–	+
Lytanthus salicinus (Lam.) Wettst.	+	–	–

* Also contains quercetin.
† Also contains scutellarein 6,4′-dimethyl ether, and the 6-methyl ether and 6,4′-dimethyl ether of 6-hydroxyluteolin. Both fresh and herbarium tissue examined.

indeed, ubiquitous in the group. Luteolin and scutellarein are occasionally present and the flavonol quercetin was detected in a single species.

In view of the discovery of 6-hydroxyluteolin throughout the family, a more detailed study was made of flavones in the leaf of a European species *G. cordifolia* in order to determine their glycosidic form. 6-Hydroxyluteolin was found to be present as the 7-glucoside, identified by direct comparison with material from *Catalpa* leaf,[6] and as the 7-diglucoside. More detailed examination also revealed the presence in this plant of scutellarein, present as the 7-diglucoside, and of three methylated flavones. One of these was identified as pectolinarigenin (scutellarein 6,4′-dimethyl ether) (III) by direct comparison with material from *Kerria* (see below), and the other two as the 6-monomethyl (V), and the 6,4′-dimethyl ethers (VI) of 6-hydroxyluteolin. 6-Methoxyluteolin (nepetin or eupafolin) has been previously reported in *Nepeta hindostana* (Labiatae),[11] *Eupatorium cuneifolium*[12] and *Centaurea*

[9] R. TIEMANN, *Arch. Pharm.* **241**, 289 (1903).
[10] A. WUNDERLICH, *Arch. Pharm.* **246**, 257 (1908).
[11] N. R. KRISHNASWARMY, T. R. SESHADRI and P. J. TAHIR, *Indian J. Chem.* **6**, 676 (1968).
[12] S. M. KUPCHAN, C. W. SIGEL, R. J. HEMINGWAY, J. R. KNOX and M. S. UDAYAMURTNY, *Tetrahedron* **25**, 1603 (1969).

arguta[13] (both Compositae) and direct comparison with material from the latter source confirmed the identification. The 6,4′-dimethyl ether of 6-hydroxyluteolin has only been reported once before, in aerial parts of *Centaurea nigrescens* and *C. phrygia*,[14] and no direct comparison was possible; however, the properties of the material from *Globularia* agreed in every respect with those reported earlier for this compound.

Flavones of the Labiatae

Although much work has been done on the flavones in this large family (200 genera, 3000 species), 6-hydroxyluteolin has not been recorded before. However, its 6-methyl ether has recently been found in *Nepeta*[11] and scutellarein is well represented in the family, especially in *Scutellaria*. Scutellarein is also known as its 7-monomethyl and 7,4′dimethyl ether in *Galeopsis ladanum*,[15] as its 6,7-dimethyl ether in *Scrophularia grossheimii*[16,17] (Scrophulariaceae), as its 6,7,4′-trimethyl ether in *Salvia triloba*[18] and as its tetramethyl ether in *Marrubium peregrinum*.[19] Earlier surveys[20,21] have also established that in the Labiatae flavones such as luteolin are common, and that flavonols such as quercetin are restricted to a few genera such as *Lamium*[22] and *Prunella*.

The present survey of 40 species from 33 genera (Table 2) revealed the presence, as expected, of 6-hydroxyluteolin in eight species belonging to the tribes Prostantheroideae, Ajugoideae or Stachyoideae. In the five species of the Ajugoideae, it was accompanied by the isomeric 8-hydroxyluteolin, hypolaetin. In view of the ready, irreversible conversion under acid conditions of hypolaetin to 6-hydroxyluteolin, it is possible that the naturally occurring glycosides in these plants are derived from the 8- rather than the 6-isomer. Hypolaetin is only known previously in the monocotyledonous family Restionaceae,[23] so that its presence in the dicotyledons, in the Labiatae, needs confirming by more rigorous procedures and this is in progress.

The present survey has confirmed that luteolin is widespread in the Labiatae and a number of new records of its occurrence in the family have been established (see Table 2). The flavonols, kaempferol and quercetin, have also been detected in a number of new sources, but they are still uncommon in the family as a whole. Methylated flavones are regular constituents in the family and their further study would clearly be very rewarding from the systematic point of view.

Flavones of the Plantaginaceae

The Plantaginaceae, a small family of 3 genera and 200 species, is not included in the order Tubiflorae in Engler's Syllabus,[24] but is generally recognized[25] as being closely allied

13 J. L. B. FUNES, B. M. MARRERO and A. G. GONZALEZ, *An. Quim.* **64**, 187 (1968).
14 F. BOHLMANN and C. ZDERO, *Tetrahedron Letters* (33) 3239 (1967).
15 K. GRITSENKO and V. I. LITVINENKO, *Khim. Prir. Soed.* **5**, 55 (1969).
16 S. G. AKHMEDOV and V. I. LITVINENKO, *Khim. Prir. Soed.* **5**, 54 (1969).
17 S. G. AKHMEDOV, *Azerb. Khim. Zh.* **80**, (1968).
18 A. ULUBELEN, S. OZTURK and S. ISILDATICI, *J. Pharm. Sci.* **57**, 1037 (1968).
19 L. A. SALEY, D. P. PAPA and G. V. LAZUREVSKII, *Khim. Prir. Soed.* **5**, 182 (1969).
20 L. HÖRHAMMER and H. WAGNER, in *Chemistry of Natural and Synthetic Colour Matters* (edited by T. S. GORE), pp. 315–330. Academic Press, London (1962).
21 R. HEGNAUER, *Chemotaxonomie der Pflanzen.*, Vol. IV, Birkhauser-Verlag, Basel (1966).
22 J. B. HARBORNE, *Phytochem.* **6**, 1569 (1967).
23 J. B. HARBORNE and H. T. CLIFFORD, *Phytochem.* **8**, 2071 (1969).
24 A. ENGLER, *Syllabus der Pflanzenfamilien*, Vol. II, 12th Edn. (edited by H. Melchior), Borntraeger, Berlin (1964).
25 R. HEGNAUER, in *Perspectives in Phytochemistry* (edited by J. B. HARBORNE and T. SWAIN), pp. 121–138, Academic Press, London (1969).

TABLE 2. A FLAVONE SURVEY OF THE LABIATAE

Subfamily, genus and species	Flavone detected in leaf
Prostantheroideae	
Westringia fruticosa Druce	Luteolin and 6-Hydroxyluteolin
Hemigenia purpurea R. Br.	
Ajugoideae	
Trichostema lanatum Benth.	6- and 8-Hydroxyluteolin, (also usually luteolin)
T. lanceolatum Benth.	
T. dichotomum L.	
Amethystea caerulea L.	
Isanthus caeruleus Mx.	
Ocimoideae	
Hyptis pectinata Poit.	No flavones detected
H. radiata L.	
Ocimum basilicum L.	
Aeolanthus parvifolius Benth.	
Prasiodeae	
Prasium majus L.	Luteolin, methylated flavone
Scutellarioideae	
Salazaria mexicana Torr.	Methylated flavone
Lavanduloideae	
Lavandula spica L. var *pyrenaica* Benth.	Luteolin
L. pedunculata Cav.	Luteolin
L. multiflora L.	None
Stachyoideae	
Dracocephalum thymiflorum L.	Luteolin
D. austriacum L.	Quercetin, kaempferol
Melittis melissophyllum L.	Luteolin
Eremostachys laciniata L.	Methylated flavones
Phlomis lychnitis L.	Luteolin
P. fruticosa L.	Luteolin
Wiedemannia erythrotriche Benth.	Quercetin, kaempferol
Moluccella spinosa L.	Apigenin
Monarda fistulosa L.	Luteolin and methylated flavones
Blephila hirsuta Benth.	Methylated flavone
Sphacele calycina Benth.	6-Hydroxyluteolin
Ziziphora hispanica L.	Luteolin
Hedeoma drummondi Benth.	Luteolin & methylated flavone
Saccocalyx satureiodes Coss et Dr.	Luteolin
Pogogyne douglasii Benth.	Luteolin, quercetin
Ceranthera linearifolia Ellis	Quercetin
Monardella odoratissima Benth.	Luteolin
Bystropogon canariensis L'Her.	Methylated flavone
Cunila marina L.	
Preslia cervina Opiz	
Collinsonia canadensis L.	Luteolin, quercetin
Elsholtzia cristata Willd.	Methylated flavone
Dysophylla auricularia Blume	Luteolin
Micromeria juliana (L.) Benth.	Luteolin

TABLE 3. FLAVONE SURVEY OF THE PLANTAGINACEAE

Plantago species		Leaf flavone pattern
P. acanthophylla Decne. *P. algarbiensis* Sampano *P. alpina* L. *P. argentea* Chaix* *P. barbata* Forst. f.† *P. major* L.‡ *P. maritima* L.*	*P. radicata** *P. subulata* L. *P. uniglumis* Wallr. ex Walpers	6-Hydroxyluteolin and Luteolin
P. amplexicaulis Cav. *P. braziliensis* Cab. & Fab. *P. fuscescens* Jord. *P. haussknechtii* Vatke *P. indica* L. *P. lagopus* L.* *P. lanceolata* L.	*P. monosperma* Poir. *P. montana* Lam.* *P. nivalis* Boiss. *P. notata* Lag. *P. ovata* Forsk. *P. serraria* L. *P. tenuiflora* Waldst & Kit.	Luteolin
P. bellardi All. *P. cretica* (L.) Boiss.		Quercetin§

* Both fresh and herbarium specimens examined. In the case of *P. maritima*, a range of fresh material of different populations were examined and while there were significant quantitative variations in leaf flavones, the qualitative pattern was uniform.

† Also contained scutellarein.

‡ Contained scutellarein and hispidulin instead of 6-hydroxyluteolin and luteolin. Hispidulin was also tentatively identified in *P. haussknechtii*.

§ Present in *P. bellardi* as rutin (confirmed by co-chromatography).

to those families within the order which are now shown to have 6-hydroxyluteolin. Examination of 26 species of the genus *Plantago* indeed showed the presence of 6-hydroxyluteolin accompanied by luteolin in eight. Scutellarein was detected in two species, luteolin occurred alone in a further 14 species and the flavonol quercetin was detected in two species. These results show that the family, as expected, has a predominantly flavone leaf pattern and they agree with the earlier examination of four species by Bate-Smith,[1] in which he found flavonols and leucoanthocyanidins to be absent. While scutellarein and its 6-methyl ether have already been reported in the family, in leaves of *P. major* var *asiatica*[26,27] this is the first finding in it of 6-hydroxyluteolin.

Other Records of 6-*Hydroxyluteolin*

Results of surveying some other families in the Tubiflorae for flavones are shown in Table 4. Of these, only the Buddleiaceae contains 6-hydroxyluteolin as a regular feature. This is a taxon until recently placed as Loganiaceae in the order Contortae, and in the latest revision now included (see Ref. 24) as a family in this order. Turning to the Pedaliaceae, the presence of 6-methoxyluteolin is known in *Sesamum indicum*,[28] and this has been

[26] T. NAKAOKI, N. MORITA and M. ASAKI *J. Pharm. Soc. Japan* **81**, 1697 (1961).
[27] M. ARITOMI, *Chem. Pharm. Bull. Tokyo* **15**, 432 (1967).
[28] N. MORITA, *Chem. Pharm. Bull. Tokyo* **8**, 59, 66 (1960).

TABLE 4. FLAVONE SURVEY OF OTHER TUBIFLORAE FAMILIES

Family, genus and species	Leaf flavones detected
Buddleiaceae	
Chilianthus lobulatus Benth.	6- and 8-Hydroxyluteolin, acacetin
Buddleia globosa Hope	6-Hydroxyluteolin, luteolin
B. heterophylla Nootboum	Acacetin, quercetin
B. polystachya Fresen.	Luteolin, quercetin, kaempferol
B. lindleyana Fortune var. *minor*	Acacetin
Pedaliaceae	
Harpagophytum sp.	Luteolin
Sesamum indicum L.	Methylated flavones
Martynaceae	
Martynia fragrans Lind.	Luteolin
M. louisiana Mill.	Luteolin
Myoporaceae	
Eremophila latifolia F. Muell.	Unidentified flavone (dk → yell., R_f 73 Forestal)
E. sturtii R. Br.	Quercetin, kaempferol, unidentified flavone (dk → dk, R_f 32 Forestal)
E. oppositifolia R. Br.	Quercetin, kaempferol
Myoporum deserti A. Cunn.	Luteolin
M. acuminatum R. Br.	Luteolin
M. serratum R. Br.	Kaempferol
Lentibulariaceae	
Utricularia gibba subsp. *excelsior*	None detected
Phrymaceae	
Phryma leptostachya L.	None detected, but flavone present in direct extract
Loganiaceae	
Gelsemium sempervirens L.	Quercetin, kaempferol
Polypremum procumbens L.	Scutellarein (?)

confirmed in the present work. This survey of only two species of the Pedaliaceae (a family of 14 genera) was not really extensive enough to establish whether 6-hydroxylated flavones are regularly present in the family. Another probable source of such compounds is the Myoporaceae, since the 3,6,7,4′-tetramethyl ether of the flavonol 6-hydroxymyricetin has been reported in *Eremophila fraseri*.[29] However, the present limited survey failed to show 6-hydroxyluteolin, although an unidentified compound of similar colour reactions was found in one species (Table 4). Similarly, limited surveys of the Acanthaceae, in which an 8-methoxyflavone has been reported (*Andrographis*),[30] and of the Verbenaceae and Scrophulariaceae, both families with one record or more of a 6-hydroxylated flavone in methylated form,[4,16,17,31] failed to reveal the presence of either scutellarein or 6-hydroxyluteolin. These are, however, all large families mainly of tropical origin, plant material of

[29] P. R. JEFFERIES, J. R. KNOX and E. J. MIDDLETON *Australian. J. Chem.* **15**, 532 (1962).
[30] T. R. GOVINDACHARI, P. C. PARTHASARTHY, B. R. PAR and P. S. SUBRAMANIAN, *Tetrahedron* **24**, 7027 (1968).
[31] A. K. BARVA, P. CHAKRABARTI and P. K. SANYAL, *J. Indian Chem. Soc.* **46**, 271 (1969).

which is accessible with difficulty and wider surveys might well reveal these compounds to be present with some frequency. The only tropical and sub-tropical Tubiflorae family that has been substantially surveyed for leaf flavonoids is the Gesneriaceae; there are, so far, no reports of 6-hydroxylated flavones in this family, but 6- or 8- hydroxylation is almost certainly a structural feature of the desoxyanthocyanin columnin, present in the flowers of a number of gesnerads.[6,32]

A search for 6-hydroxyluteolin in orders close to the Tubiflorae revealed its presence in two sources. An examination of the Valerianaceae (11 species, 5 genera) showed the presence of luteolin in four, quercetin in two and of luteolin accompanied by 6-hydroxyluteolin in *Valerianella eriocarpa* Desv. The second source was *Coreopsis mutica* (Compositae) which contains 6-hydroxyluteolin as the 7-glucoside; this record was established in collaboration with D. J. Crawford.[33] The presence of such a flavone in the Compositae is to be expected, in view of the regular occurrence in the family of a number of 6- and 8-hydroxylated flavones and flavonols, usually in *O*-methylated form. Indeed, 6-hydroxyluteolin is already known in the family as the 6-methyl ether (*Eupatorium, Centaurea*),[12,13] 3'-methyl ether (*Mikania*),[34] 6,4'-dimethyl ether (*Centaurea*)[14] and as the 6,7,4'- and 6,3',4'-, trimethyl ethers (*Eupatorium*).[12]

Families regarded as close to the Tubiflorae apparently lacking scutellarein or 6-hydroxyluteolin are the Caprifoliaceae (11 spp./6 genera surveyed), the Adoxaceae (only known species *Adoxa moschattelina* L. surveyed) and the Dipsacaceae (10 spp./8 genera surveyed). It is much easier to establish the presence of such characters at the family level than their absence and further surveys of these families are still needed to confirm these conclusions.

6-Hydroxylated flavones seem to be generally absent from other angiosperm families, although they have not been deliberately searched for in more than a few of the less specialized plant groups. They are not reported in any of the families of the first five orders of the Sympetalae and particularly not in the Ericaceae, Primulaceae[35] or Plumbaginaceae[36] which have been adequately surveyed for such compounds. Again, they do not generally appear as flavonoids of the Archichlamydeae, although isoflavones with 6-hydroxyl substitution do occur in a few taxa of the Leguminosae.[4] Where families of the Archichlamydeae have been reasonably well studied for flavonoids, e.g. the Umbelliferae[5] and Dilleniaceae,[37] they have not been detected. However, they are not completely absent, since there is a single record of scutellarein (I) in *Sorbaria* (Rosaceae),[38] a finding confirmed independently by Plouvier[39] who found it in seven species of this genus but not in a number of related taxa. In the course of other studies, the occurrence of scutellarein 6,4'-dimethyl ether (pectolinarigenin) (III) in *Kerria japonica* was uncovered, thus making a second record of this type of compound in the Rosaceae and in the Archichlamydeae.

Attention was given to the flavonoids of *Kerria japonica* leaf, following the report by Bate-Smith[40] that the three unispecific genera in the tribe Kerrieae differed in their phenolic pattern from other members of the sub-family Rosoideae, particularly in lacking ellagic

[32] J. B. HARBORNE, *Phytochem.* **5**, 589 (1966).
[33] D. J. CRAWFORD, in press.
[34] W. HERZ, P. S. SANTHANAN, H. WAGNER, R. HOER, L. HORHAMMER and L. FARKAS, *Tetrahedron Letters* 3419 (1969).
[35] J. B. HARBORNE, *Phytochem.* **7**, 1215 (1968).
[36] J. B. HARBORNE, *Phytochem.* **6**, 1415 (1967).
[37] K. KUBITZKI, *Ber. Deut. Botan. Ges.* **81**, 238 (1968).
[38] M. ARISAWA and T. NAKAOKI, *Yagugaku Zasshi* **89**, 705 (1969).
[39] V. PLOUVIER, *C.R. Acad. Sci. Paris* **269** (Series D), 646 (1969).
[40] E. C. BATE-SMITH, *J. Linn. Soc.* **58**, 39 (1958).

acid. One of several unusual constituents occurred in *Kerria japonica*, having a dark colour in u.v. light and R_f 0·75 in Forestal solvent. This has now been identified unambiguously (see Experimental) as scutellarein 6,4′-dimethyl ether (pectolinarigenin) first identified in 1932 from *Linaria vulgaris* petals (Scrophulariaceae).[41]

Neither of the other two taxa in the Kerrieae contained pectolinarigenin, although *Rhodotypos scandens* had a dark u.v. absorbing component with a similar R_f. Luteolin was present in *R. scandens* and not apigenin as suggested by Bate-Smith.[40] *Neviusia alabamensis*, reported by him to have two dark components R_fs 0·77 and 0·86 in Forestal, in our hands gave only quercetin and kaempferol, the dark spots thus probably being due to unhydrolyzed flavonol glycoside.

DISCUSSION

The present work establishes that 6-hydroxyflavones occur characteristically in a number of plant families in the Tubiflorae and in several families in adjacent orders. These flavones are of too irregular occurrence at the species level, except perhaps in the Globulariaceae, to be reliable phenetic characters, but they do appear to be of phylogenetic interest, particularly in comparison to the distribution patterns of other 6- and 8- hydroxyflavonoids. The familial distribution pattern of 6-hydroxyflavones as at present known is shown in Table 5

TABLE 5. PLANT FAMILIES WITH 6- OR 8-HYDROXYLATED FLAVONOIDS

A. Plant Families, and genera, with 6-Hydroxyflavones*
Acanthaceae (*Andrographis*)
Bignoniaceae (*Catalpa, Tecoma*)
Buddleiaceae (*Buddleia, Chilianthus*)
Compositae (*Coreopsis, Centaurea, Eupatorium, Mikania*)
Gesneriaceae (*Columnea*)*
Globulariaceae (*Globularia, Lytanthus*)
Labiatae (*Amethystea, Isanthus, Trichostema, Hemigenia, Westringia, Sphacele, Monardella, Micromeria, Scutellaria*)
Plantaginaceae (*Plantago*)
Scrophulariaceae (*Digitalis, Scrophularia*)
Valerianaceae (*Valerianella*)
Verbenaceae (*Lippia*)
Rosaceae (*Kerria, Sorbaria*)
B. Plant Families, and genera with 8-Hydroxyflavonols†
Compositae (*Chrysanthemum*)
Crassulaceae (*Sedum*)
Empetraceae (*Ceratiola, Corema, Empetrum*)
Ericaceae (*Erica, Kalmia, Ledum, Phyllodoce, Rhododendron, Rhodothamnus*)
Leguminosae (*Acacia, Coronilla, Lotus*)
Malvaceae (*Althea, Gossypium, Hibiscus*)
Papaveraceae (*Meconopsis, Papaver*)
Primulaceae (*Dionysia, Douglasia, Primula*)
Ranunculaceae (*Ranunculus*)
Scrophulariaceae (*Mimulus*)

* Usually scutellarein, 6-hydroxyluteolin or their methylated derivatives; *Columnea* has a 6- or 8-hydroxydesoxyanthocyanin, *Andrographis* an 8-methoxyflavone.

† Gossypetin and/or herbacetin: data from Harborne,[42] but see also.[43]

[41] L. SCHMID and W. RUMPEL, *Monatsh. Chem.* **60**, 8 (1932).
[42] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).
[43] I. URSCHLER, *Phyton. Ann. Rei. Botan.* **13**, 15 (1968).

and compared with that of the structurally related 8-hydroxyflavonols, herbacetin and gossypetin, which have also been recently surveyed.[42]

The most striking feature of Table 5 is that whereas the 8-hydroxyflavonols occur, almost without exception, in angiosperm families which either retain many primitive morphological features (e.g. Ericaceae) or which are generally regarded as being primitive (e.g. Ranunculaceae),[43] the 6-hydroxyflavones are present only in highly evolved families. This suggests that in the evolutionary development in leaf flavonoid pattern from accumulation of flavonol to that of flavone, there has also been a significant change in the orientation of A-ring hydroxylation from the 8- to the 6-position. If this is so, one might expect 8-hydroxyflavones, 6,8-dihydroxyflavones and 6-hydroxyflavonols (e.g. quercetagetin) to take up an intermediate position in this scheme and certainly all that is at present known about the occurrence of these compounds fits in with this idea. 6,8-Dihydroxyflavonoids, for example, have only been recorded with any frequency in the Lauraceae (*Lindera*), Rutaceae (*Citrus*) and the Compositae.

The above scheme illustrates an evolutionary trend and some exceptions are bound to become apparent as wider surveys are developed. The exceptional occurrence of the 6-hydroxyflavone pectolinarigenin in two members of the Rosaceae has already been noted. However, this finding in no way invalidates the general conclusion, particularly as Cronquist[44] regards the Asterideae as having evolved from the Rosideae, a class which includes the Rosaceae. Again, the presence of both 8-hydroxyflavonols (gossypetin) and 6-hydroxyflavones (6-hydroxyluteolin) in the highly evolved Compositae may at first seem contradictory. However, this is not really unexpected in view of the vast size of the family and the fact that evolutionary development in morphological features within the family itself is most marked.

The present survey of 6-hydroxyluteolin and scutellarein in the Tubiflorae is also of some taxonomic interest, in that it confirms the inclusion of two families, the Buddleiaceae and the Plantaginaceae, previously separated from the order, within the group. The reasons for including the Plantaginaceae within the order near to the Scrophulariaceae have been recently discussed by Hegnauer[25] and the present discovery of 6-hydroxyluteolin in *Plantago* confirms this alliance. In conjunction with this flavonoid survey, the distribution of caffeic acid esters has also been studied and the results obtained (which will be reported separately) also fit in closely with the above taxonomic alignments.

EXPERIMENTAL

Plant Material

Most species were surveyed as dried leaf tissue from the University of Liverpool Herbarium, the sheets used being duly labelled to indicate this. Fresh plants were mainly obtained from University of Liverpool Botanic Garden and identified by Dr. J. Cullen; *Utricularia gibba* was supplied by Professor C. D. K. Cook, University of Zürich, *Plantago* material by Dr. D. M. Moore, University of Reading, *Globularia cordifolia* by Professor F. Ehrendorfer, University of Graz, and *Centaurea arguta* by Mr. D. Bramwell, University of Reading.

Methods of Flavonoid Analysis

Standard chromatographic procedures were used for examining flavonoids present in direct and acid-hydrolyzed leaf extracts;[4] the common aglycones were identified by colour reactions and chromatographic comparison in four solvents.[4] 6-Hydroxyluteolin and scutellarein, when present, were further identified by co-chromatography and spectral comparison.[6]

[44] A. CRONQUIST, *The Evolution and Classification of Flowering Plants*, Nelson, London (1968).

TABLE 6. CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF 6-HYDROXYFLAVONES AND THEIR METHYLATED DERIVATIVES

Flavone	R_f (×100) in Forestal	BAW	PhOH	15% HOAc	Colour in u.v. without and with NH_3
Scutellarein	62	66	80	06	Dark brown
6,4′-Dimethyl ether	90	86	98	20	Dark brown
6-Hydroxyluteolin	53	54	42	04	Dark brown/brown
6-Methyl ether	75	73	83	12	Brown/dull yellow
6,3′-Dimethyl ether	78	83	95	08	Brown/dull yellow
6,4′-Dimethyl ether	86	80	98	12	Dark brown/dark brown

	λ_{max} (nm) in EtOH	+NaOH	+NaOAc	+$AlCl_3$	+H_3BO_3
Scutellarein					
6,4′-Dimethyl ether	279,332	367	295*	350	334
6-Hydroxyluteolin	285,349	404		375	370
6-Methyl ether	272,349	410	275	368	375
6,4′-Dimethyl ether	275,345	388	276	360	346

* Inflection.

Flavones of Globularia Cordifolia

Acid hydrolyzed extracts of the leaves yielded five flavone aglycones, which were separated and purified by chromatography on No. 3 paper in 50% HOAc, H_2O, *n*-BuOH–HOAc–H_2O (4:1:5) and *n*-BuOH–EtOH–H_2O (4:1:2·2). The spectral and chromatographic properties of the purified flavones are shown in Table 6. 6-Hydroxyluteolin (measured mass 302·0423, $G_{15}H_{10}O_7$ required 302·0426) was identified by direct comparison with material isolated earlier from *Catalpa*.[6] The 6-methyl ether of 6-hydroxyluteolin was identified as such from its spectral and R_f properties, colour reactions and its ready demethylation to 6-hydroxyluteolin; its identity was confirmed by direct comparison with authentic material isolated from *Centaurea arguta*.[13] The 6,4′-dimethyl ether of 6-hydroxyluteolin (measured mass 330·0742, $C_{17}H_{14}O_7$ required 330·0739) underwent ready demethylation during mass spectral measurement (m.u. 316, 69 per cent intensity of parent m.u.) indicating the presence of a 6-methoxyl group, and was completely demethylated to 6-hydroxyluteolin on heating with pyridinium chloride. The presence of a 4′-methoxyl was indicated by the spectral and colour properties (see Table 6) and this was confirmed by reductive cleavage, which yielded 3-hydroxy-4-methoxyphenylpropionic acid, which was distinguished from the 3-methoxy-4-hydroxy isomer by difference in R_f on a two-dimensional TLC plate run in 6 per cent HOAc in $CHCl_3$ and 45% EtOAc in benzene and by difference in initial colours with Gibbs reagent (former blue; latter pink). It also agreed in its properties with those described for the compound isolated from *Centaurea phrygia*,[14] but direct comparison was not possible.

The other two flavone aglycones were identified as scutellarein and its 6,4′-dimethyl ether, pectolinarigenin. Scutellarein was identified by direct comparison with authentic material from *Scutellaria tournefortii*, and its 6,4′-dimethyl ether by comparison with material isolated from *Kerria* (see below) or from *Centaurea arguta*.

Direct leaf extracts of *G. cordifolia* gave five flavone glycosides, after separation and purification on paper in BAW, 15% HOAc and BEW. One was identified as 6-hydroxyluteolin 7-glucoside, by direct comparison with authentic material from *Catalpa* leaf.[6] A second was the previously undescribed 6-hydroxyluteolin 7-diglucoside (R_f 21 in BAW, 37 in PhOH, 17 in 15% HOAc; λ_{max}^{EtOH} 284, 348, $\Delta\lambda^{NaOAc}$ Onm, $\Delta\lambda^{NaOEt}$ + 39 nm, $\Delta\lambda^{H_3BO_3}$ + 18 nm) and a third as the previously undescribed scutellarein 7-diglucoside (R_f 28 in BAW, 56 in PhOH, 28 in 15% HOAc; λ_{max}^{EtOH} 284, 336, $\Delta\lambda^{NaOAc}$ Onm, $\Delta\lambda^{NaOEt}$ 44 nm, $\Delta\lambda^{H_3BO_3}$ Onm). The other two glycosides were both inseparable mixtures, probably containing respectively 7-rhamnosylglucosides and 7-glucosides of the 6-methyl ether and 6,4′-dimethyl ethers of 6-hydroxyluteolin.

Pectolinarigenin from Kerria japonica

Acid hydrolyzed leaf extract of this plant yielded a flavone with the spectral and chromatographic properties of scutellarein 6,4′-dimethyl ether as shown in Table 6. On demethylation it yielded scutellarein, via a monomethyl ether and a consideration of its spectral properties indicated that it was the 6,4′-dimethyl ether. This was confirmed by mass spectral measurement, which showed a parent ion at 314 ($C_{17}H_{14}O_6$ required 314) and a large fragment at 299 (65 per cent intensity of parent ion) which is a characteristic feature of the mass spectra of 6-methoxylated flavonoids. It was also further identified by direct comparison with authentic material from *Centaurea arguta*.

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