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COMPARATIVE BIOCHEMISTRY OF THE FLAVONOIDS—VII.

CORRELATIONS BETWEEN FLAVONOID PIGMENTATION AND SYSTEMATICS IN THE FAMILY PRIMULACEAE*

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Abstract—A survey of the flavonoids and other phenolics in leaf and flower of a hundred species, representing 18 of the 25 genera, of the Primulaceae has shown that the richest variation in pigment structures occurs in the genus *Primula*. Thus, the rare 7-*O*-methylated anthocyanin hirsutin is confined to this genus (in 30 of 41 cyanic species) and occurs in all subgenera except Auganthus, where it is replaced by malvin or malvidin 3-glucoside as the principal petal pigment. The related rosinin, earlier detected in *P. rosea* petals, has now been found in *P. clarkei* in the same section. Peonidin is only found as a principal pigment in the crimson calyces of *P. viali*. Cyanin and other cyanidin glycosides are confined to the petals of *P. chungensis*, *P. aurantiaca*, *P. cockburniana* (all section Candelabra) and *P. warshenewskiana* (section Farinosae). Delphinidin occurs as the principal petal pigment only in *P. ioessa*. Other genera of the family (*Cortusa*, *Dodecatheon*, *Cyclamen*, *Soldanella*) have anthocyanins based on malvidin, delphinidin or cyanidin. The yellow flavonol in petals of *P. vulgaris*, *P. veris* and *P. elatior* (all section Vernales) previously reported as quercetagetin has now been found to be the 8-hydroxy isomer, gossypetin. Gossypetin also occurs in yellow petals of four species of the section Sikkimenses, and in one of these, *P. alpicola*, it is accompanied by herbacetin. Gossypetin has also been detected in two closely related genera *Dionysia* and *Douglasia* but yellow flower colour elsewhere in the family (e.g. *Lysimachia*) is probably carotenoid. The simple 3′,4′-dihydroxyflavone, identified in this family for the first time as a leaf glucoside, is of biogenetic interest in relation to the production of flavone in the leaf farina of *Primula*. It is also of systematic interest, since it occurs widely in *Primula* (in 39 of 55 species) and in three related genera (*Cortusa*, *Dodecatheon* and *Dionysia*) in the subfamily Primuleae but was not otherwise detected in the family. Other new phenols of taxonomic interest in *Primula* include a new trihydroxyflavone and a substance, chionanthin, with a distinctive pink fluorescence in u.v. light, which is possibly related to 7-hydroxyflavone. Leucocyanidin, leucodelphinidin, kaempferol and quercetin occur throughout the family. By contrast, myricetin is uncommon (in 4 *Primula* and in 2 *Lysimachia* spp.) and the flavones apigenin and luteolin have only been detected so far in *Soldanella*, presumably a phyletically advanced taxon. Kaempferol and quercetin occur in *Primula* leaf and petal in combination with a range of sugars and the distribution of some of these glycosides is clearly related to classification at the subgeneric level. The very common kaempferol 3-gentiotrioside, thus, is rare in section Candelabra and absent from Sikkimenses (both subgenus Aleurita), being largely replaced by rutin, which in turn is rare in the subgenus Auganthus and absent from the subgenus Craibia.

INTRODUCTION

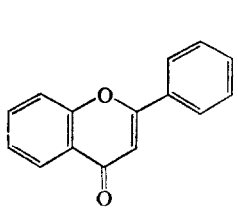
IN CONTINUING a chemotaxonomic survey of flavonoids in sympetalous families,[1–4] attention was turned to the Primulaceae, a largely temperate herbaceous group of some 25 genera and 800 species. The family occupies a relatively isolated position in most taxonomic treatments,

* Part VI—J. B. HARBORNE, *Phytochem.* **6**, 1643 (1967).

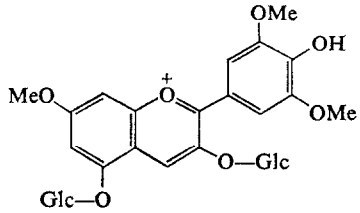
[1] J. B. HARBORNE, *Phytochem.* **5**, 11 (1966).
[2] J. B. HARBORNE, *Phytochem.* **5**, 589 (1966).
[3] J. B. HARBORNE, *Phytochem.* **6**, 1415 (1967).
[4] J. B. HARBORNE, *Phytochem.* **6**, 1643 (1967).

being usually placed on grounds of flower morphology near to the Plumbaginaceae, Theophrastaceae or Myrsinaceae.[5] By far the largest genus is the ornamentally important genus *Primula*, with perhaps 540 species, but several other genera, e.g. *Cyclamen*, *Lysimachia*, *Androsace*, are widely cultivated and material for phytochemical study is reasonably accessible.

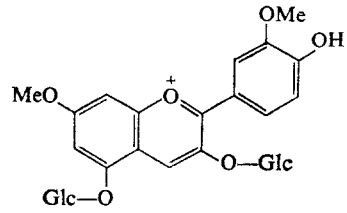
From the chemotaxonomic point of view, the Primulaceae are worth investigating since a number of uncommon flavonoids have been reported in the family.[6] Perhaps the most unusual of all is flavone itself (I), which was isolated from the farina of *Primula pulverulenta* by Muller in 1915[7] and has since been shown to be present in the farinas of about 35 other *Primula* species and in *Dionysia*.[8] The flower anthocyanins are unusual in that the 7-*O*-methylated pigment hirsutin (II) was discovered in *P. hirsuta* (now *P. rubra*) in 1927[9] and the related rosinin (III) isolated from *P. rosea* in 1960.[10,11] An uncommon yellow flower pigment quercetagetin (now shown to be gossypetin) was reported in *Primula* more recently.[12] The



Flavone (I)



Hirsutin (II)



Rosinin (III)

common flavonols kaempferol and quercetin are abundant in the family[13] but they occur, in some cases, in unusual glycosidic combination, the 3-gentiotriosides of *P. sinensis* being especially rare.[11,12]

Taxonomically, the family is divided into five tribes, two of which, the Primuleae and Lysimachieae, are further subdivided.[14] Most taxonomic problems centre round *Primula*, identification at the species level in such a large group being sometimes difficult as is the separation of *Primula* from the similar *Cortusa*, *Dionysia* and *Omphalogramma*.[15,16] In order to see if chemical data could be utilised for systematic purposes, a survey has been carried out of anthocyanins, flavonols and other phenolics, chiefly in *Primula* but also in representative species of most other genera. As a result several interesting correlations between chemistry and systematics at both species and generic level have been uncovered. In certain cases the pattern of flavonoid glycosides in the leaf and flower of *Primula* can be used for species identification. The discovery of some new simple hydroxyflavones is also reported and the opportunity has been taken to correct and expand an earlier study[12] of the yellow floral pigments.

[5] G. H. M. LAWRENCE, *Taxonomy of Vascular Plants*, p. 656. Macmillan, New York (1951).
[6] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, p. 198. Academic Press, New York (1967).
[7] H. MULLER, *J. Chem. Soc.* **107**, 872 (1915).
[8] W. C. BLASDALE, *J. Am. Chem. Soc.* **67**, 491 (1945).
[9] P. KARRER and R. WIDMER, *Helv. Chim. Acta* **10**, 758 (1927).
[10] J. B. HARBORNE, *Nature* **187**, 140 (1960).
[11] J. B. HARBORNE and H. S. A. SHERRATT, *Biochem. J.* **78**, 298 (1961).
[12] J. B. HARBORNE, *Phytochem.* **4**, 647 (1965).
[13] E. C. BATE-SMITH, *J. Linn. Soc. (Botany)* **58**, 39 (1962).
[14] H. MELCHIOR (editor), *Engler's Syllabus der Pflanzenfamilien* (12th Ed.), Vol. II. Borntraeger, Berlin (1964).
[15] W. W. SMITH and G. FORREST, *Notes Roy. Botan. Garden Edin.* **16**, 1 (1928).
[16] P. WENDELBO, *Arb. Univ. Bergen, Mat.-Nat. Ser.* No. 3, 1–89, No. 11, 1–49, No. 19, 1–31 (1961).

RESULTS

The present survey of the Primulaceae was carried out mainly on fresh leaf and flower of plants growing at the University of Liverpool Botanic Garden, material being collected when plants were in flower. Some 100 species were available for study, the plants being derived mainly from spontaneous seed or from stock of known origin. The 55 species of *Primula* examined were fully representative of the genus in terms of flower colour and varied from deep blue, mauve, red and pink to yellow and white. They were also reasonably representative morphologically, species of 17 of the 31 sections being examined, including farinose, efarinose, involute and revolute species. The survey was supplemented in the case of genera other than *Primula* by leaf material of herbarium specimens. Flavonoid identifications were based on direct spectral and chromatographic comparisons with authentic pigments. Results of surveying the plants for anthocyanin, yellow flavonols, flavones and flavonol glycosides are considered separately.

Petal Anthocyanins

The results of surveying 43 species of *Primula* with cyanic flowers are presented in Table 1, and considerably extend earlier studies of the anthocyanins of several genetically variable species.[6,11] Genetic variation can be troublesome from the taxonomic standpoint but fortunately flower colour variation is only pronounced in a few species of the subgenus Craibia, e.g. *P. sinensis*, and in several Candelabra such as *P. burmanica*; the pigments shown in Table 1 indicate, in nearly all cases, that to be found in petals of "wild type" material.

For simplicity, only the major pigments of each species are given in Table 1. Hirsutin, for example, is sometimes accompanied by traces of malvin and petunin. Direct acid hydrolysis of petals usually yields, in addition, delphinidin and/or cyanidin but a study of the glycosides has shown that these two anthocyanidins are nearly always derived from leucoanthocyanidins present in petal tissue. Only the corolla pigments have been systematically surveyed since leaf anthocyanin is only present in quantity in a handful of species (for a detailed study of leaf pigmentation in *P. sinensis*, see Ref. 11). The only pigments studied other than those of the corolla, were the anthocyanins of the striking scarlet calyx of *P. viali*. These were identified as 3-glycosides of pelargonidin and peonidin, two aglycones which are confined elsewhere in *Primula* to colour mutants of *P. sinensis*.

This survey of anthocyanin pigmentation (Table 1) clearly divides the genus into two groups, those species with 7-*O*-methylated anthocyanins and those without. Hirsutin (II) is almost universal to *Primula*, having been detected in 67 per cent of species with coloured petals. It is present in every section, with the exception of those in the subgenus Craibia (see below). The ability to synthesize 7-*O*-methylated anthocyanins may be present in several more species than those listed in Table 1. Thus, both hirsutidin and rosinidin have been detected in acid-hydrolysed extracts of some colour forms of *P. polyanthus*, a hybrid of two yellow flowered species *P. veris* and *P. vulgaris*.[6,10] Again, hirsutidin occurs in some garden forms of both *P. auricula* and *P. japonica*,[6] and may be present in wild populations. However, both these latter species hybridise readily with other *Primula* and the gene for 7-*O*-methylation may equally well have been introduced from a neighbouring species.

Rosinin (III), the related pigment to hirsutin in the cyanidin series, is clearly rare. Previously found[10,11] in rose pink petals of *P. rosea* it has now been detected in only one other species with similar petal colour, *P. clarkei*. The rarity of rosinin has been useful in correcting the name of a *Primula* plant growing at the University Botanic Gardens, collected on one of Furze's Afghanistan expeditions, which morphologically seemed to belong to the *rosea*

TABLE 1 DISTRIBUTION OF FLAVONOIDS IN THE GENUS *Primula*

Subgenus, section and species	Anthocyanins				Yellow pigments and flavones				Flavonol glycosides and cinnamic esters				Other constituents
	1	2	3	4	5	6	7	8	9	10	11	12	
SPHONDYLIA													
verticillata													
P. × *kewensis* Hort.	–	–	–	–	–	+	+	–	+	+	–	+	
AURICULASTRUM													
auricula													
P. minima L.	+	–	–	–	–	–	–	–	+	–	+	–	
P. auricula L.	–	+*	–	–	–	–	+	–	+	–	–	–	Myricetin
P. rubra I.F. Gmel	+	–	–	–	–	–	–	–	+	–	–	–	
P. marginata Curtis	+	–	–	–	–	–	+	–	–	–	+	+	
P. glaucescens Moretti	+	–	–	–	–	–	–	–	+	–	+	–	
PRIMULA													
vernales													
P. veris L.	–	–	–	–	+	+	–	–	+	+	–	–	
P. vulgaris Hudson	–	–	–	–	+	–	+	–	+	–	–	–	
P. elatior (L.) Hill	–	–	–	–	+	–	+	–	+	+	–	–	
megasaefolia													
P. megasaefolia Boiss.	+	–	–	–	–	–	–	–	–	+	+	+	
AUGANTHUS													
sinensis													
P. sinensis Sabine	–	+*	–	–	–	–	–	–	+	–	+	+	Dihydro-flavonols
malacoides													
P. malacoides Franch	–	+	–	–	–	–	+	–	+	–	+	+	
cortusoides													
P. polyneura Franch	–	+	–	–	–	–	+	–	+	+	+	+	
P. saxatilis Komarov	–	+	–	–	–	–	+	–	+	–	+	+	
obconica													
P. obconica Hance	–	+	–	–	–	–	+	–	+	–	+	+	
CRAIBIA													
petiolares													
P. whitei W. W. Sm	+	+	–	–	–	–	+	–	+	–	–	–	
P. gracilipes Craib	+	–	–	–	–	–	+	–	+	–	–	–	
P. boothii Craib	+	–	–	–	–	–	+	–	+	–	–	–	
ALEURITA													
candelabra													
P. beesiana Forrest	+	+	–	–	–	–	+	+	+	+	–	+	
P. burmanica Balf. f. et Ward	+	+	–	–	–	–	+	+	+	+	+	–	
P. aurantiaca W. W. Sm et Forrest	–	–	–	+	–	+	+	+	–	+	+	–	
P. bulleyana Forrest	+	+	–	–	–	–	+	+	–	+	–	+	Pulveruletin
P. pulverulenta Duthie	+	+	–	–	–	–	+	+	–	+	+	+	Pulveruletin
P. smithiana Craib	–	–	–	–	–	+	–	+	–	+	–	+	Myricetin
P. helodoxa Balf f.	–	–	–	–	–	+	–	+	–	+	–	+	
P. cockburniana Hemsl.	–	–	–	+	–	+	+	+	–	+	–	+	
P. chungensis Balf. f et Ward	–	–	–	+	–	–	+	+	–	+	+	–	
P. japonica A. Gray	–	+*	–	–	–	–	+	+	–	+	+	–	Pulveruletin
P. anisodora Balf. f. et Forrest	+	–	–	–	–	–	–	+	+	+	–	+	
P. prolifera Wall	–	–	–	–	–	+	–	+	–	+	–	+	
sikkimenses													
P. alpicola Stapf	+	–	–	–	+	–	+	+	+	+	–	–	Herbacetin
P. florindae Ward	–	–	–	–	+	–	+	+	–	+	–	+	
P. toessa W. W. Sm	–	–	–	–	–	–	+	–	+	+	–	–	Delphinidin

TABLE 1—(*continued*)

Subgenus, section and species	Anthocyanins				Yellow pigments and flavones				Flavonol glycosides and cinnamic esters				Other constituents
	1	2	3	4	5	6	7	8	9	10	11	12	
P. secundiflora Franch	−	+	−	−	−	−	−	+	−	+	−	−	Myricetin
P. sikkimensis Hook.	−	−	−	−	+	−	+	+	−	−	−	+	
P. waltonii Watt	+*	+*	−	−	+	−	+	+	−	+	−	+	
nivales													
P. chionantha Balf f. et Forrest	−	−	−	−	−	+	+	(+)	+	+	+	−	
P. rusbyii Greene	+	−	−	−	−	−	+	−	+	−	+	+	
P. sinopurpurea Balf f.	+	−	−	−	−	−	+	−	+	+	+	+	
farinosae													
P. farinosa L.	+	−	−	−	−	−	+	+	+	+	−	−	
P. scotica Hook	+	−	−	−	−	−	+	+	+	+	−	−	
P. frondosa Janka	+	−	−	−	−	−	+	−	+	+	−	+	
P. halleri J. F. Gmel	+	−	−	−	−	−	+	−	+	−	−	−	
P. involucrata Wall	+	−	−	−	−	−	−	−	+	+	+	−	
P. luteola Ruprecht	−	−	−	−	−	+	+	−	+	+	−	−	
P. rosea Royle	−	−	+	−	−	−	−	−	+	+	−	−	
P. warshenewskiana Fedtsch	−	−	−	+	−	−	−	−	+	−	−	+	
P. yargonensis Petitm	+	−	−	−	−	−	−	−	+	+	+	+	
P. clarkei Watt	−	−	+	−	−	−	+	−	+	−	−	+	
denticulata													
P. denticulata Smith	+	−	−	−	−	−	+	−	+	−	+	−	
capitata													
P. capitata Hook.	+	−	−	−	−	−	+	−	+	+	−	−	Myricetin
soldanelloideae													
P. cawdoriana Ward	+	−	−	−	−	−	+	−	+	+	−	+	
P. nutans Franch	+	−	−	−	−	−	+	−	+	+	+	+	
P. reidii Duthie	−	−	−	−	−	−	+	−	+	+	−	−	
muscarioides													
P. vialı Delavay ex Franch	+	−	−	−	−	−	+	−	+	−	−	−	Peonidin in calyx

Key: 1, hirsutin; 2, malvin; 3, rosinin; 4, cyanin; 5, gossypetin; 6, carotenoids; 7, 3′,4′-dihydroxyflavone; 8, chionanthin; 9, kaempferol 3-gentiotrioside (as petal pigment); 10, quercetin 3-rutinoside (as leaf pigment); 11, caffeic esters; 12, *p*-coumaric esters.

* indicates that anthocyanin is present as a 3-glucoside (or other 3-glycoside) instead of the usual 3,5-diglucoside.

group and was labelled *aff. rosea*. Examination of the anthocyanin showed that it contained, not the expected rosinin, but cyanin. Cyanin in fact occurs in *P. warshenewskiana*, another pink petalled species in the same section and this discovery suggested that the plant collected by Furze was related to *warshenewskiana*, not *rosea*. Cyanin could of course arise in *P. rosea* by mutation at the anthocyanin methylating locus. This possibility was however ruled out by a chromatographic comparison of the other flavonoids and phenols in the petals of the taxa concerned. While *P. aff. rosea* and *P. warshenewskiana* gave identical chromatographic profiles, *P. rosea* differed from the first two taxa in at least five phenolic characters.[17]

As can be seen in Table 1, hirsutin is only absent from the one subgenus Craibia of *Primula*, in which it is replaced, in all five species examined, by malvidin 3,5-diglucoside (4 species)

[7] J. B. HARBORNE, in *Chemotaxonomy and Serotaxonomy* (edited by J. G. HAWKES) p. 195. Academic Press, New York, (1968).

or malvidin 3-glucoside. This result supports Wendelbo[16] in placing species of four different sections in the same subgenus and also fits the earlier cytological analysis of the group by Bruun.[18] 7-*O*-Methylated anthocyanins are also absent from six of the *Primula* species examined in the subgenus Aleurita. The presence of cyanin instead of rosinin in *P. warshenewskiana* has already been noted. Cyanin or cyanidin 3-glycosides are also present in three related species of section Candelabra, namely *P. chungensis*, *P. cockburniana* and *P. aurantiaca*. It may be significant that in the two latter species, orange-yellow flower colours are based on an admixture of cyanidin with carotenoid, which suggests that natural selection may have operated in these species to produce a red instead of the usual blue colour due to a change in the animal pollinators of the species involved. If such were so, then the absence of anthocyanin methylation is understandable. Finally, two species in the section Sikkimenses, *P. secundiflora* and *P. ioessa*, are also anomalous in lacking hirsutin and have petal anthocyanins based on malvidin and delphinidin respectively.

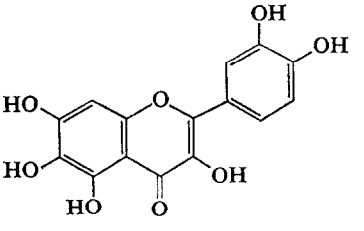
It is interesting that the majority of *Primula* species with 7-O-methylation contain hirsutidin as the 3,5-diglucoside, the related 3-glucoside being detected in but one species, *P. waltonii*. Conversely, over half the species lacking 7-*O*-methylation also lack 5-*O*-glucosylation in *Primula*, which suggests that the genes controlling these two separate steps in anthocyanin synthesis may be closely linked in this genus.

One final point of systematic interest in that 7-*O*-methylated anthocyanins, so characteristic of the genus *Primula*, have yet to be detected elsewhere in the Primulaceae. Thus, Lawrence *et al.*[19] in an earlier survey, found only pelargonidin and malvidin glycosides in *Anagallis* (3 species) and *Dodecatheon integrifolia*.

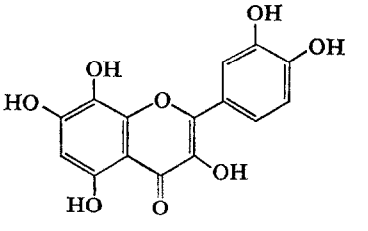
Again, van Bragt[20] reported malvin in all of 13 species of *Cyclamen* he investigated, and Wiering and de Vlaming[21] found 3- and 3,5-glycosides of malvidin, delphinidin and pelargonidin in colour forms of *Anagallis arvensis*. In the course of the present survey, species of four other genera were examined (Table 2) all with negative results. Thus, the common malvin was noted in petals of *Dodecatheon* and *Cortusa*, cyanidin 3-glycosides in *Androsace* and delphinidin 3-glucoside in *Soldanella*.

Yellow Flower Pigments

Earlier,[12] yellow flavonol glycosides based on quercetagetin (IV) were reported as principal petal pigments in three species of the Vernales section, *P. vulgaris*, *P. veris* and *P. elatior*, and in three of their hybrids *P. polyanthus*, *P.* × *sinopurpurea* and *P.* × *silva taroucana*. During the present work it has become clear that the earlier identification of IV was incorrect and that the pigments are in fact glycosides of the isomeric flavonol, gossypetin (V).



Quercetagetin (IV)



Gossypetin (V)

[18] H. G. BRUUN, *Symbolae Botan. Upsalienses* **1**, 1 (1932).
[19] W. J. C. LAWRENCE, J. R. PRICE, G. M. ROBINSON and R. ROBINSON *Phil. Trans. Roy. Soc.* **230**, 149 (1939).
[20] J. VAN BRAGT, *Mededel. Landbouwhogeschool Opzoekingstat. Staat. Gent.* **62**, 1 (1962).
[21] H. WIERING and P. DE VLAMING, private communication.

TABLE 2. DISTRIBUTION OF FLAVONOIDS IN THE PRIMULACEAE, EXCLUDING *Primula*

Subfamily, genus and species	Anthocyanins			Flavonols				Flavones		Others
	1	2	3	4	5	6	7	8	9	
LYSIMACHIEAE										
Lysimachia cuspidata Blume	−	−	−	−	+	−	−	−	−	
L. fortunei Max	−	−	−	−	−	−	−	−	−	
L. stricta Soland.	−	−	−	−	+	+	−	−	−	
L. thyrsiflora L.	−	−	−	−	+	+	−	−	−	
**L. vulgaris* L.	−	−	−	+	+	+	−	−	−	
L. nummularia L.	−	−	−	+	+	+	−	−	−	
L. atropurpurea L.	−	−	−	−	+	+	−	−	−	
Steironema heterophyllum Rafin	−	−	−	−	+	−	−	−	−	} Glycoflavones
S. ciliatum L. (Raf)	−	−	−	−	+	+	−	−	−	
S. radicans A. Gray	−	−	−	−	+	+	−	−	−	
Trientalis americana Pursl.	−	−	−	−	+	+	−	−	−	
T. europea L.	−	−	−	−	+	+	−	−	−	
Asterolinum stellatum (L.) Hoffgg & Lk	−	−	−	−	+	+	−	−	−	
Glaux maritima L.	−	−	−	−	+	+	−	−	−	
Anagallis arvensis L.	−	−	−	−	+	+	−	−	−	Pelargonidin and malvidin 3-glucosides
A. linifolia L.	−	−	−	−	+	+	−	−	−	
Centunculus minimus L.	−	−	−	−	+	−	−	−	−	
CYCLAMINEAE										
Cyclamen graecum Link.	−	−	−	+	+	+	−	−	−	
PRIMULEAE										
**Cortusa matthioli* L.	+	−	−	−	+	+	−	+	−	
**Androsace lactea* L.	−	−	−	−	+	+	−	−	−	Rutin
**A. lanuginosa* Wall.	−	−	+	−	+	−	−	−	−	
**A. obtusifolia* All.	−	−	−	−	+	+	−	−	−	
**A. sarmentosa* Wall.	−	−	+	−	+	+	−	−	−	Rutin and unidentified flavonol
**A. Sempervivoides* Jacquem.	−	−	+	−	+	−	−	−	−	
**A. villosa* L.	−	−	−	−	+	+	−	−	−	
**Douglasia vitaliana* B & H	−	−	−	−	+	−	+	−	−	Rutin and unidentified flavonol
**Dionysia aretioides* (Lehm.) Boiss.	−	−	−	−	+	+	+	+	−	
D. revoluta Boiss.	−	−	−	−	+	+	−	+	+	
D. diapensiifolia Boiss.	−	−	−	−	+	+	−	+	+	
D. bryoides Boiss.	−	−	−	−	−	−	−	+	−	
Dodecatheon meadia L.	+	−	−	−	+	+	−	+	−	Rutin and kaempferol gentiotrioside
D. cusickii Greene	−	−	−	−	+	+	−	+	−	
D. clevelandi Greene	−	−	−	−	+	−	−	+	−	
Hottonia palustris L.	−	−	−	−	−	+	−	−	−	
**Soldanella alpina* L.	−	+	−	−	+	+	−	−	−	Rutin
**S. montana* Mikan.	−	+	−	−	+	+	−	−	−	{ luteolin & apigenin
**S. reticulata*	−	+	−	−	+	+	−	−	−	
S. pusilla Baumg.	−	−	−	−	−	−	−	−	−	{ luteolin and apigenin
S. carpatica Vierh.	−	−	−	−	−	−	−	−	−	
S. hungarica Simk.	−	−	−	−	−	−	−	−	−	

TABLE 2—(*continued*)

Subgenus, genus and species	Anthocyanins			Flavonols				Flavones		Others
	1	2	3	4	5	6	7	8	9	
SAMOLEAE										
Samolus valerandi L.	–	–	–	–	–	+	–	–	–	
CORIDEAE										
Coris monspelliensis L.	–	–	–	–	+	+	–	–	–	
Coris hispanica Lange	–	–	–	–	+	+	–	–	–	

Key: 1, malvin; 2, delphinidin 3-glucoside; 3, cyanidin 3-glycoside(s); 4, myricetin; 5, quercetin; 6, kaempferol; 7, gossypetin; 8, 3′,4′-dihydroxyflavone; 9, chionanthin.
* indicates fresh material available; other results on herbarium tissue.

Only milligram amounts of pigment were available for study in the earlier work and the distinction between IV and V was based on colour and chromatographic differences between quercetagetin isolated from *Tagetes erecta*[22] and a sample of gossypetin supplied by Professor T. R. Seshadri. The identification was confirmed by reductive acetylation and hydrolysis to yield an anthocyanidin (6-hydroxycyanidin) identical to that obtained from quercetagetin. Since then, a second sample of gossypetin became available, isolated from *Chrysanthemum segetum* and of proven structure.[23] Reductive acetylation and hydrolysis of this also gave an anthocyanidin indistinguishable from 6-hydroxycyanidin,[24] so that isomerism of the 8- to the 6-hydroxycyanidin must occur during the hydrolysis step.[25] This result showed that the original identification was open to question. The *Chrysanthemum* gossypetin was found to have similar colour properties on paper (dull black) and identical R_f values to quercetagetin, so that the earlier distinction between the isomers was no longer valid.

Comparisons of the u.v. and i.r. spectral properties of the two isomers (see Table 3 and experimental) now show that the two flavonols can be readily distinguished on a micro scale by spectral means. Both the neutral and alkaline absorption spectra (the latter measured in the presence of sodium borohydride[26]) are significantly different; in addition, gossypetin gives a blue colour (λ_{max} 625 nm) with sodium acetate not shown by quercetagetin (λ_{max} in EtOH-NaOAc 381 nm). Re-examination then of the flavonol aglycone from *P. vulgaris* petals showed it to be gossypetin and not quercetagetin and similar re-examination of yellow flavonols from *Rhododendron*, *Papaver* and *Lotus* claimed to be quercetagetin in the earlier investigation[12] also showed them to be based on gossypetin. The major flavonol glycoside of *P. vulgaris* petals must therefore now be formulated as gossypetin 3-gentiotrioside.

During the re-investigation of the *P. vulgaris* flavonol aglycones, a second component with the same colour reactions as gossypetin but of higher R_f in all solvent systems was noticed. A better source of this new pigment was found in the petals of the yellow form of *P. alpicola*, where it occurs as the major pigment with gossypetin as the minor constituent. This new pigment is identical with mimuletin earlier isolated[12] from yellow petals of *Mimulus luteus* and thought at the time to be 6-hydroxykaempferol. It is now clear from a study of

[22] A. G. PERKIN, *J. Chem. Soc.* **103**, 209 (1913).
[23] T. A. GEISSMAN and C. STEELINK, *J. Org. Chem.* **22**, 946 (1957).
[24] H. M. HURST and J. B. HARBORNE, *Phytochem.* **6**, 1111 (1967).
[25] L. JURD and J. B. HARBORNE, *Phytochem.* **7**, 1209 (1968).
[26] H. A. SCHROEDER, *Phytochem.* **6**, 1589 (1967).

TABLE 3. CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF *Primula* FLAVONOLS AND FLAVONES

Flavonoid	Source(s)	R_f (×100) in BAW	Forestal	PhOH	5% HOAc	10% HOAc† —$CHCl_3$	Colour in u.v. (+NH_3)
Flavonols:							
Quercetagetin	*Tagetes* petal	31	26	12	00	00	Dull black (dull brown)‡
Gossypetin	*Chrysanthemum* petal and *Primula vulgaris*	31	26	12	00	00	
Herbacetin	*P. alpicola* petal *Mimulus luteus* petal	59	42	45	00	00	
Flavones:							
3′,4′-Dihydroxy	*P. pulverulenta* leaf and synthetic	80	80	95	06	24	Blue (yellow)
Pulveruletin	*P. pulverulenta* leaf	68	68	90	06	06	
7,3′,4′-Trihydroxy	Synthetic	68	68	88	05	10	
Chionanthin	*P. chionantha* farina	92	90	97	14	60	Pink (yellow)
7-Hydroxy	Synthetic	90	87	98	08	42	

Spectral maxima (in nm)

	95% EtOH	EtOH/NaOEt§	EtOH/NaOAc	EtOH/$AlCl_3$	EtOH/H_3BO_3
Flavonols:					
Quercetagetin	260, 274, 365	273, 405	283, 381	425	Unstable
Gossypetin	264, 277, 338, 386	272, 435	252, 370, 625	370, 449	Unstable
Herbacetin	258, 276, 330, 380	435	275, 370, 625	371, 444	Unstable
Flavones					
3′,4′-Dihydroxy	218, 245, 309, 343	294, 422	No shift	No shift	367
Pulveruletin	228, 247, 341	290, 393	No shift	No shift	368
7,3′4′-Trihydroxy	237, 255,* 314*, 344	258, 408	254, 267*	No shift	370
Chionanthin	214, 250, 294, 307*	250, 296, 307*, 406		No shift	No shift
7-Hydroxy	255, 277, 308	269, 310*, 366		No shift	No shift

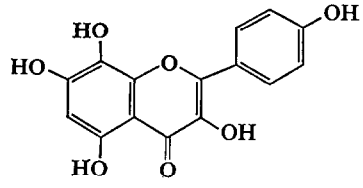
*=inflection.

† on silica gel, others on Whatman no. 1 paper.

‡ when run in phenol, gossypetin differs from quercetagetin in appearing dull yellow instead of black.

§ Alkaline spectra of the flavonols were measured in aqueous solution in presence of sodium borohydride (λ_{max} of quercetagetin in H_2O 258, 272, 357 nm: of gossypetin 260, 272, 335, 375 nm. $\Delta\lambda$ alk are thus +48 and +60 nm respectively).

its spectral and other properties that it is 8-hydroxykaempferol (VI), the known pigment herbacetin.[27]



Herbacetin (VI)

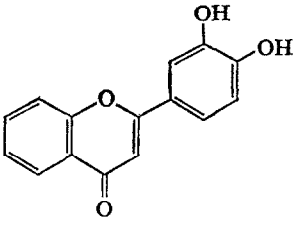
[27] K. NEELAKANTAN and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **4**, 54 (1936).

A third yellow flavonol in *P. vulgaris*, designated as Primula F 3 A and originally thought[12] to be a dimethyl ether of quercetagetin, must now be formulated as a dimethyl ether of gossypetin. Apart from the fact that it gives a small, possibly anomalous, borate shift in its spectrum it could be the as yet unknown 8,4′-dimethyl ether, but further structural studies are required to confirm this. It is probably isomeric with tambuletin, originally formulated as 8-methoxykaempferol[28] but more recently shown to be a dimethyl ether of gossypetin, possibly the 7,3′-dimethyl ether.[29]

As can be seen from Table 1, gossypetin is a taxonomic marker of some significance in the genus *Primula*. Thus, besides occurring throughout the section Vernales, it has now been found in all species of the section Sikkimenses with yellow flowers, namely in *P. alpicola*, *P. florindae*, *P. sikkimensis* and *P. waltonii*. It is, however, completely absent from the neighbouring section, Candelabra, where yellow flower pigmentation is based on carotenoid pigments. Presence of gossypetin as a flower pigment also links *Primula* with the closely related genera *Dionysia* and *Douglasia* (see Table 2) and it is significant that this flavonol could not be detected in a more distant genus with yellow flowered species, namely *Lysimachia*.

Leaf and Farina Flavones

In the course of a chromatographic examination of the leaf hydrolysates of *Primula* species, an unknown substance was detected with high R_f in *n*-butanol–acetic acid–water and with a blue fluorescence in u.v. light, changing to yellow with ammonia. Spectral examination (Table 3) suggested that it was a flavone, confirmed by examination of the spectrum of its acetate, which was identical to that of flavone itself (I). Mass spectral analysis showed that it was a dihydroxyflavone and the facts that it has a green $FeCl_3$ colouration and gives a borate shift in its spectrum suggests that it is 3′,4′-dihydroxyflavone (VII), hitherto unknown as a natural product. Detailed comparison (see Experimental) with synthetic material confirmed this identification. VII does not occur in *Primula* leaf in the free state, since it appears on chromatograms of direct extracts as a dull mauve spot, its colour unchanged by ammonia, which gives VII and glucose on hydrolysis. The colour, spectral properties and R_fs of the glycoside suggest that VII is present as the 4′-glucoside.



3′,4′-Dihydroxyflavone (VII)

3′,4′-Dihydroxyflavone is accompanied in leaf of *Primula bulleyana*, *P. japonica* and *P. pulverulenta* by a related compound, pulveruletin, of similar colour properties and spectrum but of lower R_f and with a molecular weight corresponding to a trihydroxyflavone. The only known flavone with these properties is 7,3′,4′-trihydroxyflavone, recently isolated from *Trifolium*[30] but direct comparison of the *Primula* compound with synthetic material showed

[28] K. A. BALAKRISHNA and T. R. SESHADRI, *Proc. Indian Acad. Sci.* **25**A, 449 (1947).
[29] P. LEBRETON and J. B. HARBORNE, unpublished results.
[30] A. L. LIVINGSTON and E. M. BICKOFF, *J. Pharm. Sci.* **53**, 1557 (1964).

that they were similar but not identical. Another likely structure on biogenetic grounds is 3′,4′,5′-trihydroxyflavone, and syntheses of this and other trihydroxyflavones are in progress for comparison with the *Primula* substance.

3′,4′-Dihydroxyflavone is a particularly good chemical marker in having a distinctive fluorescence and R_f and it is easy to score it in leaf hydrolysates of plants. A survey showed that it was of universal occurrence in *Primula*, being present in 71 per cent of the species (Table 1) examined and in practically every section of the genus. It was also found in three closely related genera, *Dionysia*, *Dodecatheon* and *Cortusa* (Table 2) but no trace could be detected in any species of 14 other genera of the Primulaceae examined.

Another systematically interesting flavone was detected in acid hydrolysates of flower extracts. This substance, called chionanthin, has a very high R_f (i.e. indicating few hydroxyl groups) and a very distinctive pink fluorescence in u.v. light, changing to yellow with ammonia. It is only matched in R_f and colour reactions amongst simple flavones by 7-hydroxyflavone, but it differs significantly in spectral properties (Table 3). Spectral analysis, indeed, suggests that chionanthin is possibly a mixture of flavone with several monohydroxyflavones, but attempts to separate chionanthin into constituents have so far failed.

Although the structure of chionanthin is still not clear, its value as a systematic marker is on firmer ground. It is confined as a petal constituent almost entirely to the sections Candelabra and Sikkimenses of the subgenus Aleurita. Its only other occurrences in *Primula* are in *P. farinosa* and in *P. scotica* (section Farinosae) of the same subgenus. It also occurs in the farina of many of the above plants and has also been detected in the farina of *P. chionantha*, section Nivales, but again of the same subgenus.

The survey of *Primula* farinas has been handicapped by the facts that production is variable and that it is often only present on the stem or in the calyces round the seed pod. Spectral and TLC analysis of some 20 species confirms that flavone itself is the major constituent. The other two substances reported in farinas—5-hydroxyflavone in *P. imperialis*[31] and 5,8-dihydroxyflavone (primetin) in *P. modesta*[32]—are both pale yellow and must be rare since the majority of farinas are white. Indeed, only one species with intense yellow farina was available for examination, *P. chionantha*. Examination by TLC showed that the farina is a mixture of at least five phenolic constituents. One is chionanthin and a second corresponds in R_f and colour to 5-hydroxyflavone, but was not present in sufficient amount for spectral confirmation. A major constituent, yellow in visible light with λ_{max} 282 and 366 nm and showing a positive $AlCl_3$ shift, may be primetin but lack of material for comparative purposes has so far prevented confirmation of this identification. Further studies of the farinas of this and other *Primula* species are in progress.

Flavonol Glycosides and Cinnamic Acid Esters

All *Primula* leaf material examined gave kaempferol and/or quercetin on hydrolysis. In addition, myricetin was obtained from leaf of four species in the subgenus Aleurita, *P. capitata*, *P. ioessa*, *P. secundiflora* and *P. smithiana*. Petal tissues of most species similarly yield the same two flavonols and myricetin was obtained as petal constituent of just one species, *P. auricula*. A few species of the section Candelabra were exceptional in that the flowers either lacked flavonols completely (e.g. *P. japonica*, *P. pulverulenta*) or contained only traces (e.g. *P. chungensis*).

[31] P. KARRER and G. SCHWAB, *Helv. Chim. Acta* **24**, 297 (1941).
[32] W. NAGAI and S. HATTORI, *Chem. Z.* **11**, 409 (1930).

Two dimensional paper chromatography of leaf and flower extracts of *Primula* showed that these flavonols occur as a wide range of glycosides, with as many as ten glycosides in some species. The pattern of spots on the chromatograms was often species-specific, particularly if other phenolic constituents were taken into account as well. Chromatography could probably be used directly in taxonomic work for separating species, particularly in those cases where clear cut morphological differences are not available. For example, the three species examined of the section Petiolares are chromatographically distinctive. *P. whitei* can be separated from *P. boothii* by five species-specific leaf characters and one petal character, *P. gracilipes* being similarly separated by four leaf and two petal characters. Again, *P. helodoxa* and *P. prolifera*, two morphologically similar species in the section Candelabra, differ from each other in leaf chemicals. They have four constituents in common, but *P. helodoxa* has two and *P. prolifera* six species-specific phenols. There has been no opportunity during the present survey to test for interspecific variation in *Primula* but the indications are that flavonoid characters are reasonably stable in this respect.

Two glycosides, kaempferol 3-gentiotrioside and quercetin 3-rutinoside (rutin), were run as markers on all two-dimensional chromatograms and their distribution is recorded in Table 1. Kaempferol 3-gentiotrioside, isolated earlier from petals of *Primula sinensis*,[11] is clearly characteristic of the genus, being present in 73 per cent of the species as a petal constituent. It is also widely distributed in the leaves. Similarly, rutin earlier isolated from *P. chionantha* leaf[6] and now obtained in quantity from *P. pulverulenta*, is widespread as a leaf constituent (present in 64 per cent of the species). The distribution of both glycosides follows the established taxonomy to some extent. Thus, rutin is rare or absent from the subgenera Craibia, Auganthus and Auriculastrum, but by contrast is very common in the subgenus Aleurita (e.g. present in all twelve Candelabra species). Again, kaempferol 3-gentiotrioside, present in every species (19/19) of the majority of sections of the subgenus Aleurita, is uncommon (5/18) in sections Candelabra and Sikkimenses and marks them off from the rest of the subgenus.

Judging from R_f values, three other glycosides present in *P. sinensis* flowers,[11] namely quercetin 3-gentiotrioside and kaempferol and quercetin 3-gentiobiosides, are fairly widely distributed in the genus. Opportunity is taken here to report the confirmation of the structure of the quercetin 3-gentiobioside of *P. sinensis* by comparing it with material synthesized in Professor Wagner's laboratory in Munich. Kaempferol 3-rutinoside is almost certainly present in a number of species but it has yet to be positively identified. Two incompletely characterized 3-glycosides of kaempferol and quercetin respectively, occurring particularly in species of the subgenus Petiolares, are unusual in having high R_f values in aqueous solvents. They do not match any of the many known 3-glycosides and appear to have the sugars glucose and rhamnose in some new di- or trisaccharide combination.

The flavonoid pattern outside *Primula* is very similar in that kaempferol and quercetin are commonly present in most other genera of the family. Rutin, reported in 13 *Cyclamen* species by van Bragt,[20] has now been detected in *Androsace* and *Dodecatheon* (Table 2). Only two genera were distinctly different from *Primula; Lysimachia*, in having myricetin in several species, and *Soldanella*, in which luteolin and apigenin appear to replace kaempferol and quercetin in several species. Leucoanthocyanidins, so commonly distributed in *Primula* leaf and flower that no attempt was made to score species for them, are also found widely in other genera; *Soldanella* is perhaps the only genus free of them. Dihydroflavonols such as dihydrokaempferol, which have been found in *P. sinensis* during the earlier genetic studies,[11] have not been detected in quantity elsewhere in the genus or the family.

The only common phenolic constituents not mentioned so far are hydroxycinnamic acid esters; present surveys show they are widespread in the group. The distribution of *p*-coumaric and caffeic acid esters in *Primula* is recorded in abbreviated form in Table 1 (+ indicates the presence in *either* leaf *or* flower, − indicates absence from *both* organs). From R_f values, it is clear that some five different esters of both *p*-coumaric and caffeic acids occur in the same genus and they may be present in considerable quantities in either leaf or petal or both tissues of the majority of species. The form the combination takes is not clear but they are resistant for the most part to acid hydrolysis and do not therefore correspond to any of the common sugar or quinic acid esters. As taxonomic markers they are of limited interest, except at the species level. There is, however, some correlation between their occurrence and subgeneric classification. *p*-Coumaric and caffeic esters are thus uniformly present in Auganthus, but uniformly absent from Craibia and from Primula (except *P. megaesifolia*). Otherwise, they occur, but not consistently, in most species of the other three subgenera.

DISCUSSION

An exhaustive discussion of the bearing of present flavonoid data on the systematics of the Primulaceae is clearly not warranted here, because coverage in terms of numbers of species has perforce been limited. Only 55 of some 550 described *Primula* species have been available for examination and coverage at the sectional level, even, has been variable. Some sections could not be analysed because the species, native to the Himalayas, have never survived when brought into cultivation in this country and are only known as herbarium specimens. Complete ascertainment at the family level has not been achieved, only about three quarters of the genera having been analysed and some of these superficially. Again, only a few of the more interesting flavonoid characters have been scored and much remains to be done in studying the flavonol glycosidic patterns of *Primula* and other genera.

Three main points of systematic interest do however emerge from the chemical results. First, the difficulties encountered by taxonomists in drawing generic limits based on morphology in the subfamily Primuleae are clearly reflected in the chemistry. Thus several chemicals characteristic of *Primula*, e.g. gossypetin and 3′,4′-dihydroxyflavone, overlap into very similar genera such as *Dionysia*, *Douglasia*, *Dodecatheon* and *Cortusa*. *Dionysia* is particularly difficult to separate from *Primula* and Wendelbo[16] has found that the division can only be based on multi-assessment of morphological characters; chemically there is no distinction, apart from the apparent absence of hirsutin from *Dionysia*. Wendelbo considers that *Dionysia* could equally well be placed in *Primula* as an isolated subgenus and the chemical data would fit such an arrangement. The absence of 3′,4′-dihydroxyflavone from *Androsace* (7 species examined) is noteworthy, since it is a genus morphologically close to *Primula*, and this may contribute a useful distinguishing feature between the genera.

The second main point worth making is that the chemistry adds useful support to Wendelbo's recent reclassification of the many *Primula* species into seven subgenera. The subgenus Auganthus, for instance, can be separated on four chemical characters: presence of malvidin glucoside, caffeic and *p*-coumaric esters in the petals and absence of rutin from the leaves. The subgenera Craibia, Primula and Auriculastrum all have some chemical points of distinction (see Table 1). Aleurita is the largest subgenus and chemical characters here are not completely consistent; nevertheless it separates from other subgenera in having chionanthin. The two sections Candelabra and Sikkimenses of Aleurita have been reasonably well covered species-wise and it is interesting to compare their chemistry. They are united in having

chionanthin but differ in their flavonol glycosides and in their yellow pigmentation. The yellow gossypetin of Sikkimenses replaces the more usual yellow carotenoid coloration of Candelabra. Gossypetin here may be regarded as a "chemomimetic" character in the same way as the yellow 3-hydroxykynurenine replaces pteridine-based yellow pigments in the wings of certain butterfly genera.[33]

A third point of systematic interest is that chemistry confirms the Primulaceae as a family rather isolated from any of its neighbours in the Sympetalae. The simple flavones present in *Primula* have not been detected anywhere else in the plant kingdom. Likewise, 7-*O*-methylated anthocyanins are very rare, and have only been found once elsewhere, in *Lochnera*[34] (Apocynaceae). Again, gossypetin does not appear as a yellow flower pigment in any related families, the nearest record being in *Rhododendron* (Ericaceae).

The two families usually linked with the Primulaceae in most classifications are the Plumbaginaceae and the Myrsinaceae but both these groups have very different flavonoid patterns. The Plumbaginaceae, which has been studied in some detail,[3] is characterized by 5-*O*-methylated flavonoids, plumbagin, myricetin 3-rhamnoside and the aurone cernuoside as a yellow flower pigment. Less is known of the woody Myrsinaceae but a survey of ten species from four genera failed to reveal any of the characteristic flavonoids of the Primulaceae. Leaves contained myricetin, quercetin and kaempferol, and ellagic acid (recorded only once in the Primulaceae, in *Hottonia palustris*[13]) is rather common (in three *Myrsine* and in *Ardisia pickeringia*).

The most interesting substance discovered during the present survey is 3′,4′-dihydroxyflavone, since it forms a biogenetic link between the flavone of *Primula* farina and the common flavonol quercetin of *Primula* leaf. Its presence in the leaf sap in glycosidic form indicates that the unusual biosynthetic pathway which operates in *Primula* to give flavone and simple flavones lacking either A- or B-ring hydroxyls is not confined to the leaf surface. There are indications that the farinas in some species of *Primula* contain complex mixtures of simple hydroxyflavones and their analysis may well throw further light on the biosynthetic origin of the simple flavones in this genus.

EXPERIMENTAL

Plant Material

Fresh petals and leaves were collected from plants growing at the University of Liverpool Botanic Gardens at Neston, Cheshire. Identification was made by the Director, J. K. Hulme and further verification is being made by Dr. J. Cullen, who has made voucher specimens of all the living plant material. Material of *Primula farinosa*, *P. minima*, *P. rubra*, *Douglasia vitelliana* and *Androsace obtusifolia* were kindly provided by J. K. Hulme, from collections made in the Italian Alps in the summer of 1967. Nomenclature of *Primula* follows that of Smith and Wright, except that *P. scotica* is retained as a separate species, instead of a subspecies of *P. farinosa* (c.f. [35]). *P. rusbyii* is retained in Table 1 in the Nivales section although it is also included by some authorities in the section Parryii. Three forms (or subspecies) of *P. alpicola* were examined with violet, yellow and white petals and the results shown in Table 1 summarize the flavonoid results from all these forms. Dried leaf material was taken from plants in the University of Liverpool herbarium.

General Survey for Flavonoids

Leaf and flower were hydrolysed separately by heating in 2N HCl for 30 min at 100° and the aglycones were extracted into ethyl acetate (flavonols, flavones and cinnamic acids) and amyl alcohol (anthocyanidins). The aglycones were identified by paper chromatography in *n*-butanol–acetic acid–water (4:1:5) (BAW). Forestal, 50 per cent HOAc and PhOH-H_2O against standard markers. 1 per cent MeOH-HCl extracts of coloured petals were examined for anthocyanins by chromatography in BAW, Bu-HCl, 1 per cent aq. HCl

[33] K. S. BROWN, *Systematic Zool.* **16**, 213 (1967).
[34] W. G. C. FORSYTH and N. W. SIMMONDS, *Nature* **180**, 247 (1957).
[35] D. MCCLINTOCK and R. S. R. FITTER, *The Pocket Guide to Wild Flowers*, p. 125. Collins, London (1956).

and HOAc-HCl-H_2O (15:3:82) and pigments were identified by co-chromatography with standard markers. Concentrated EtOH extracts of leaf and flower of all fresh plants were run 2-dimensionally on paper in BAW and 5 per cent HOAc, with rutin and kaempferol 3-gentiotrioside as markers.

Identification of Gossypetin in Primula vulgaris

Fresh primrose petals were heated in 2N HCl for 30 min under N_2, the cooled extract was extracted into ethyl acetate and the crude flavonoid material so obtained was chromatographed on Whatman No. 3 paper in 50 per cent HOAc. The major flavonoid band, yellow in visible light and dull black in u.v. light, was eluted, purified by chromatography in BAW, and obtained as a yellow powder. It was compared with quercetagetin, isolated from *Tagetes erecta* (mol.wt. by mass spectra 318, required value 318) and with gossypetin, prepared by acid hydrolysis under N_2 from a sample of gossypitrin kindly supplied by Professor C. Steelink. It was identical in every respect to the gossypetin sample and differed from quercetagetin in u.v. spectral properties (Table 3), i.r. spectrum and in giving a blue colour with NaOAc and a purple red colour in the "gossypetone" test. Infrared spectra were determined in KBr discs on material dried at 80°/½ mm for 3 hr. Quercetagetin was readily distinguished from gossypetin and quercetin by the presence of three very intense absorption bands at 9·25, 9·75 and 10·45 μ. By contrast, gossypetin had bands of medium intensity in this region at 9·5, 10·1 and 10·3 μ, while quercetin had weak bands at 9·2 and 9·95 μ. Quercetagetin and gossypetin could not be distinguished chromatographically either on paper or on layers of silica gel, cellulose and polyamide using a wide range of solvents. The nature of the substance provided by Professor T. R. Seshadri and thought earlier[11] to be gossypetin is not clear but it could be a methylated derivative of a flavonol more highly hydroxylated than gossypetin.

Isolation and Identification of Herbacetin from Primula alpicola

Fresh petals were heated in 2 N HCl for 30 min under N_2, and the crude flavonoid, obtained by extraction into ethyl acetate was purified by chromatography on No. 3 paper in 50 per cent HOAc and BAW. A minor constituent, of lower R_f, separating during the chromatography in 50 per cent HOAc, was identified as gossypetin (see above). The major aglycone was obtained as a yellow EtOH-soluble powder, m.p. 244° (decomp.). Its molecular weight by mass spectra was 302 (required for pentahydroxyflavone 302). It was clearly 8-hydroxykaempferol (herbacetin) and not 6-hydroxykaempferol since it gave a blue colour with NaOAc and a wine red "gossypetone" reaction (c.f. gossypetin); furthermore in its spectral properties, it was very close to gossypetin but distinctly different from quercetagetin. On reductive acetylation, followed by acid hydrolysis, it gave a pigment indistinguishable from aurantinidin (6- or 8-hydroxypelargonidin). It was spectrally and chromatographically identical to mimuletin, obtained earlier[11] from *Mimulus luteus* petals, and with a minor flavonol constituent of *P. vulgaris*, which separated during chromatography from the gossypetin present.

Identification of 3′,4′-Dihydroxyflavone and Isolation of a Trihydroxyflavone

Leaf of *P. pulverulenta* was heated for 30 min at 100° in 2N HCl and the ethyl acetate extract was chromatographed in BAW. Elution of the strong blue fluorescent band (R_f 0·88) gave crude material, which mass spectral analysis showed to be a mixture of dihydroxyflavone (main peak at 254 nm) and trihydroxyflavone (minor peak at 270 nm). The dihydroxyflavone was obtained pure by chromatography in 50 per cent HOAc and had the spectral properties shown in Table 3. It gave a green colour with $FeCl_3$. The acetate had λ_{max} 255 and 297 nm and was unusually unstable in alkali, ring opening to give the corresponding chalcone (alk. max 306 and 405 nm). It was identical spectrally and chromatographically to synthetic material prepared by Baker-Venkataraman re-arrangement of the veratroyl ester of *o*-hydroxyacetophenone. The 3′,4′-dimethoxyflavone so obtained (pale yellow needles from EtOH, m.p. 154° lit. m.p. 154–5°, λ_{max} 213, 242, 312 and 333 nm) was demethylated to 3′,4′-dihydroxyflavone by heating in pyridinium chloride for 4 hr to give 3′,4′-dihydroxyflavone as colourless plates from EtOH, m.p. 246° (lit. m.p. 243°).[36] Chromatographic comparison of the natural and synthetic material included a number of thin-layer systems besides the one mentioned in Table 3. 3′,4′-Dihydroxyflavone, both the natural and synthetic specimens, behaved anomalously on reductive cleavage and failed to yield any of the expected 3,4-dihydroxyphenylpropionic acid.[24] Instead, three catechol derivatives were formed as the major degradation products, of R_fs 0·16/0·44, 0·29/0·58 and 0·45/0·58 on silica gel in 10 per cent HOAc–$CHCl_3$ and 45 per cent EtOAc–C_6H_6 respectively. These substances are presumably, from their R_fs and colour reactions, derivatives of 2′,3,4-trihydroxydihydrochalcone and of the related alcohol. 3′,4′-Dihydroxyflavone was isolated from direct leaf extract of *P. pulverulenta* as the 4′-glucoside(?) R_f 0·63 in BAW and 0·21 in 5 per cent HOAc. It had a dull mauve colour in u.v. light, unchanged by fuming with ammonia, and gave glucose and 3′,4′-dihydroxyflavone on acid hydrolysis. It had λ_{max}^{EtOH} 242, 256, 310 and 332 nm and $\lambda_{max}^{EtOH-EtONa}$ 286 and 385 nm.

A novel trihydroxyflavone, pulveruletin, was obtained as a minor blue fluorescent band of lower R_f than 3′,4′-dihydroxyflavone, after re-chromatography of the *pulverulenta* leaf extract in 50 per cent HOAc. Pulveruletin was very similar to a synthetic sample of 7,3′,4′-trihydroxyflavone but could be distinguished from

[36] FRASCHINA BERNSTEIN, and S. VON KOSTANECKI, *Chem. Ber.* **38**, 2180 (1905).

it by minor differences in the u.v. spectrum (Table 3) and by a small difference in its colour properties and the fact that these two compounds separated when chromatographed on silica gel in 10 per cent HOAc-$CHCl_3$. Pulveruletin was also different from 7,3′,4′-trihydroxyflavone in the products it gave on reductive cleavage.

Chionanthin and Other Flavones from P. chionantha *farina**

Mass spectral analysis of the crude pale yellow farina showed the presence of some monohydroxyflavone (M238) together with much parent flavone (M 222). Separation on silica gel in 10 per cent HOAc-$CHCl_3$ gave 5 bands. The first band (R_f 0·92) was dull brown in u.v. light and was identical in R_f and colour reactions to 5-hydroxyflavone. Spectral analysis, λ_{max} 257, 295, 310 (inflection) nm, showed it was contaminated with very large amounts of flavone, which does not itself give any colour in u.v. light on silica gel. The second band (R_f 0·68) (primetin?) was yellow in visible light, dark in u.v. light. It had λ_{max}^{EtOH} 282, 366; $\lambda_{max}^{EtOH-AlCl}$ 296, 360 nm; $\lambda_{max}^{EtOH-EtONa}$ 290, 350 nm and gave no shift with either borate or acetate. It gave a strong blue colour with the Folin-Ciocalteu reagent, prior to treatment with NH_3, typical of hydroquinone and catechol derivatives. The third band (R_f 0·60, chionanthin) was pink in u.v. light, changing to yellow with NH_3. It had spectral properties shown in Table 3. It was identical to material isolated from *P. pulverulenta* flowers. Two minor phenolic bands (R_fs 0 84 and 0·52) present in the farina extract were too weak for spectral analysis.

Flavonol glycosides of Primula

A sample of kaempferol 3-gentiotrioside isolated from *P. sinensis* during earlier studies, was available for comparative studies. The structure of quercetin 3-gentiobioside, also obtained earlier from *P. sinensis*, was confirmed by comparing the natural with synthetic material kindly provided by Professor H. Wagner. Co-chromatography on paper in six solvent systems showed that the pigments were identical.

Rutin was isolated as pale yellow needles, after chromatography of *P. pulverulenta* leaf extracts, and recrystallized from water. It was identified by R_f, u.v. and i r. spectral comparison with authentic material. The partly characterized glycosides K1 and Q1 were isolated from leaf of *P. heladoxa*; K1 had R_fs 0·35 and 0·71 and Q1 R_fs 0·27 and 0·69 in BAW and water respectively. K1 had λ_{max} 266 and 348 nm and gave all the spectral shifts with inorganic ions characteristic of kaempferol 3-glycosides; similarly, Q1 had λ_{max} 256 and 356 nm and gave the shifts characteristic of quercetin 3-glycosides. Both gave glucose and rhamnose on acid hydrolysis. Comparison with R_f data for most of the known glycosides of kaempferol and quercetin (Ref 6, pp 69–70) showed that K1 and Q1 do not correspond with known pigments.

At least five *p*-coumaric esters were detected as colourless–blue spots in u.v. light on chromatograms of *Primula* species. R_f values (× 100) in BAW and 5 per cent HOAc and typical sources were as follows: 91/75,83 *P. anisodora* leaf, 85/67,84 *P. prolifera* petal, 70/76,81 *P. polyneura* leaf and petal, 61/83 *P. marginata* petal and 52/66,83 *P cockburniana* leaf. When compared with *p*-coumaroylquinic acid (73/74,84) in other solvents, the ester 85/67,84 had the following values: 02(14) in butanol–2 N NH_3 (1:1) and 61(59) in BuOH–EtOH–H_2O (4:1:2·2). Some of the caffeic esters in *Primula* had the following R_fs in BAW and 5 per cent HOAc: 77/67,78 (*P. megaesifolia* leaf), 70/58,72 (*P. pulverulenta* leaf), 61/70,79 (*P. saxatilis* leaf and petal), 51/70,77 (*P. saxatilis*) and 35/57,77 (*P. chungensis* leaf).

Survey of Leaf Flavonoids in the Myrsinaceae

Herbarium leaf (or fresh leaf in the case of *Ardisia*) was hydrolysed in 2 N HCl at 100° for ½ hr and the flavonoids detected in the usual way. None of the characteristic *Primula* constituents (e.g. 3′,4′-dihydroxyflavone) was detected. The results were as follows: *Ardisia crenata* Sims. ardisiin (unidentified phenol[37] with strong mauve fluorescence in u.v. light); *A. crispa* A.D.C. ardisiin; *A. polycephala* Wall. ardisiin, myricetin, quercetin, kaempferol, leucodelphinidin; *A. wallichi* A.D.C. myricetin, leucodelphinidin and three ardisiin-like compounds; *A. pickeringia* Torr. & Gray ex DC. myricetin, quercetin and ellagic acid; *Maesa sinensis* A.D.C. quercetin; *Myrsine africana* Linn. myricetin and unidentified pigment R_f 0·80 in BAW, dark brown in visible light; *M. capitellata* Wall. ellagic acid; *M. melanophlores* R. Br. quercetin and ellagic acid; *M. kellau* Hochst. myricetin, quercetin, kaempferol and ellagic acid; *Rapanea variabilis* Mez. myricetin, quercetin, kaempferol, leucoanthocyanidin.

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[37] J. B. HARBORNE in *Methods in Polyphenol Chemistry* (edited by J. B. PRIDHAM), p. 32. Pergamon Press, Oxford (1964).

* The presence of 5,8-dihydroxyflavone (primetin) in *P. chionantha* farina has now been confirmed by chromatographic comparison with *P. modesta* farina[32] material [added in proof].