were recrystallized from aqueous ethanol containing 7% conc. HCl. Details of their identification are summarized in Table 1. Hydrolysis, partial hydrolysis of the anthocyanins and identification of their hydrolysis products were performed as described earlier.[8] The pigments II, III and IV were monoglycosides since controlled hydrolysis gave only two spots, (the aglycone and the unchanged pigment). The $FeCl_3$ test was carried out according to Leon, *et al.*[1] Only pigment II was decolourized (less than 30 min).

Acknowledgement—We wish to thank Professor Arne Fredga for his kind interest in this work and for collecting the plant material.

TABLE 1. IDENTIFICATION OF ANTHOCYANINS OF *Nymphaea candida*

	Absorption spectra			*After total acid hydrolysis		R_f values (× 100) in.†			
Band	λ_{max} (nm)		$\frac{E_{440}}{E_{vis.\ max}}$	Aglycone	Sugar	A	B	C	D
I	531,	283	0·22	Cyanidin	Glucose	34	33	03	27
II	543,	281	0·18	Delphinidin	Galactose	35	35	18	39
III	530,	283	0·23	Cyanidin	Galactose	36	24	07	27
IV	541,	278	0·17	Delphinidin	Galactose	24	10	03	17

* In MeOH containing 0·01% conc. HCl.
† Determined by PC on Whatman No. 1.
A = *n*-BuOH–HOAc–H_2O (4:1:5, by vol., top layer);
B = *n*-BuOH–2N HCl (1:1, v/v top layer);
C = HCl–water (3:97, v/v);
D = HOAc–HCl–water (15:3:82, by vol.)

[8] G. BENDZ and Å. HAGLUND, *Acta Chem. Scand.* **21**, 2286 (1967).

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PRIMULACEAE

HIRSUTIN AND GOSSYPETIN IN *DIONYSIA*

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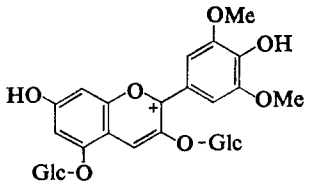
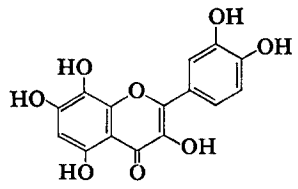
Abstract—An earlier flavonoid survey of the Primulaceae has now been extended to a further eight *Dionysia* and seven *Primula* species. As a result, hirsutin has been identified for the first time outside *Primula* as the violet pigment in flowers of *Dionysia archibaldii*, *D. bryoides*, D. *curviflora* and *D. microphylla*. Gossypetin, earlier found as a yellow pigment in flowers of *D. aretioides* is now reported also in *D. bornmuelleri* and *D. paradoxa*. Other characteristic *Primula* markers, namely flavone in the farina and 3′,4′-dihydroxyflavone in the leaf, were present in all species now examined. These results bring the total number of chemical characters of *Primula* present in *Dionysia* to five and correlate with the close morphological relationship known to exist between the two genera. The presence of primetin in the farina of *P. chionantha* has now been confirmed.

INTRODUCTION

Dionysia contains 38 species, 13 of which have violet or pink flowers and 25 yellow flowers.[1] It is a phytochemically interesting genus because of its close relationship with *Primula* and it shares with *Primula* the unique ability to produce flavone as a farina-type secretion on the leaves and stems. *Dionysia* plants, however, are generally inaccessible since they are native to Afghanistan, Iran and Turkey, and many species have only recently been discovered and brought into cultivation.[2,3] During a previous survey of flavonoids in the Primulaceae,[4] only four species were examined and only one, a yellow flowered species, in the living state. Chemical comparison showed that *Dionysia* was very similar to *Primula* and a study of anthocyanins in the genus seemed particularly desirable, since most violet-flowered *Primula* species contain, as an important taxonomic marker, a unique anthocyanin pigment, hirsutin, the 3,5-diglucoside (I) of the 7-*O*-methylated anthocyanidin hirsutidin.



(I) Hirsutin



(II) Gossypetin

Through the courtesy of Professor Per Wendelbo and Mr. J. C. Archibald, fresh material of seven *Dionysia* species and dried material of an eighth became available for phytochemical analysis and the results are presented here. At the same time, further *Primula* species have also been examined, some of which belong to the subgenus of *Primula* most closely allied to *Dionysia*.

RESULTS

The results of the present survey are shown in Table 1. The most significant finding is that of the pigment hirsutin in four *Dionysia* species. This is the first report of hirsutin outside *Primula*. The four species containing hirsutin in the flowers individually represent all three of Wendelbo's sections within the genus[2] and this suggests that hirsutin could well be present in all of the 13 known violet-flowered *Dionysia* species.

Chromatographic examination of flower extracts of *D. archibaldii*, *D. bryoides*, and *D. curviflora* also showed that they were identical in having three flavonol glycosides in common.

The yellow flavonoid gossypetin (II), earlier reported in flowers of *Dionysia aretioides*[4] has now been found in two further yellow-flowered species, namely *D. bornmuelleri* and *D. paradoxa*. This finding again suggests that yellow flower colour in *Dionysia* is characteristically based on yellow flavonols rather than on carotenoids. In *Primula*, gossypetin and related compounds have only been found so far in species of the sections Primula (syn. Vernales) and Sikkimensis. The only other known source of gossypetin in the Primulaceae is

[1] P. WENDELBO, *Bot. Notiser* **120**, 144 (1967); **123**, 300 (1970).
[2] P. WENDELBO, *Arb. Univ. Bergen, Mat. Nat. Ser. No.* 3, 1–89 (1961).
[3] P. WENDELBO, *Arb. Univ. Bergen, Mat. Nat. Ser. No.* 19, 1–31 (1963).
[4] J. B. HARBORNE, *Phytochem.* **7**, 1215 (1968).

TABLE 1. FLAVONOIDS IN LEAVES AND PETALS OF *Dionysia* AND *Primula* SPECIES

Plant species	Source*	Farina flavone	Leaf flavonoids† 1	2	3	4	Petal pigments
Dionysia aretioides (Lehm.) Boiss.	GB (P.W. 1959)	+	+	+	+	+	Gossypetin
D. bornmuelleri (Pax) Clay	GB (Rech. 11485)	+	+	+	+	−	Gossypetin
D. paradoxa Wendelbo	GB (Lamond 1913)	n.d.	+	+	+	+	Gossypetin
D. teucrioides Davis et Wendelbo	GB (C.M.&W. 3619B)	+	+	+	+	−	n.d.
D. microphylla Wendelbo	GB (P.W. 8363)	+	+	−	+	+	Hirsutin
D. archibaldii Wendelbo	J.C.A. 3010	n.d.	n.d.	n.d.	n.d.	n.d.	Hirsutin
D. bryoides Boiss.	J.C.A. 2906	n.d.	n.d.	n.d.	n.d.	n.d.	Hirsutin
D. curviflora Bunge	J.C.A. 2800	n.d.	n.d.	n.d.	n.d.	n.d.	Hirsutin
Primula boveana Dcne.	GB (Danin 1968)	+	+	+	+	+	n.d.
P. gaubaeana Born.	J.C.A. 2647	+	+	+	+	+	n.d.
P. macrocarpa Maxim.	GB (Jap. exp. 52/57)	+	+	+	+	+	n.d.
P. rotundifolia Pall.	GB (Ghose 1967)	+	+	+	+	+	n.d.
P. verticillata Forsk.	GB (Arnst. 1955)	+	+	+	+	+	n.d.
P. yuparensis Takedo	GB (Sapporo 56/79)	+	+	+	+	+	n.d.
P. aureata Fletcher	GB (S.S.W.9322)	+	+	+	+	−	n.d.

* GB indicates that the plant is in cultivation at the Botanic Garden of Göteborg, Sweden, and the number in parenthesis is the reference source. J.C.A. numbers refer to plants from J. C. Archibald.

† Key: 1,3′,4′-dihydroxyflavone; 2, chionanthin; 3, quercetin; 4, kaempferol; + present; −, absent; n.d., not determined.

Vitaliana primulifera (syn. *Douglasia vitaliana*, cf. Ref. 4) a genus closely related to *Androsace* and somewhat more distantly related to *Primula* and *Dionysia*.

Three of the seven *Primula* species examined (*P. boveana*, *P. gaubaeana* and *P. verticillata*) (Table 1) belong to the subgenus Sphondylia, which according to Wendelbo,[2] is particularly closely related to *Dionysia*. Only one taxon (*P.x kewensis*) of this group was previously studied.[4] The results of analysing the flavonoids of leaf and farina were unexceptional; all contained, as expected, flavone in the farina and 3′,4′-dihydroxyflavone together with chionanthin in the leaf.

The present results show that from the point of view of flavonoid chemistry, *Dionysia* cannot be distinguished from *Primula*. Thus, all five distinctive chemical markers (flavone, 3′,4′-dihydroxyflavone, chionanthin, hirsutin, gossypetin) found characteristically in *Primula* also occur regularly in *Dionysia*. These data are correlated with the well recognised close morphological similarity between the two genera and also support the view that *Dionysia* evolved as a separate genus from within *Primula*. Since *Dionysia* is most closely related morphologically with *P. floribundae* (subgenus Sphondylia), it has been suggested that it originated from ancestral stock of these plants in the mountains of East Arabia.[2] However, the apparent absence of gossypetin, the principal yellow pigment in *Dionysia*, from plants of this subgenus does not support this idea. On the other hand, the gossypetin character links *Dionysia* with *Primula* subgenus Primula, and, indeed, these two plant groups are known to have pollen characters in common. It is possible, therefore, that the origin of *Dionysia* lies within this subgenus rather than within Sphondylia.

According to Wendelbo,[2] there are easily recognisable progressive trends in the development of morphological characters within *Dionysia*. Unfortunately the chemical results on

Dionysia are so far very uniform and do not as yet give any indication of correlations with phylogeny in the genus.

In the previous paper on the flavonoids of the Primulaceae,[4] the provisional identification of the rare farina constituent, primetin (5,8-dihydroxyflavone) in *P. chionantha* was mentioned. This identification has now been fully confirmed (see Experimental) by means of direct comparison with natural material from *P. modesta*[5] and with a synthetic sample.[6]

EXPERIMENTAL

Plant material. Plant material was provided by Professor P. Wendelbo or Mr. J. C. Archibald (as indicated in Table 1) and identified by them. It was supplied as fresh material, except for *D. microphylla*, which was dried leaves and flowers, collected by P. W. in Afghanistan from plants growing at Maimana, Darrah Abdullah near Belcheragh. Voucher specimens are held by Professor Wendelbo.

Flavonoid identifications. Flavonoids were identified in leaf, flower or farina by methods outlined earlier.[4] Hirsutin was identified in flowers of *D. microphylla* by direct chromatographic and spectral comparison with an authentic sample from *P. capitata* flowers.[4] It was further identified by acid hydrolysis to give hirsutidin. Due to shortage of material it could only be identified in flowers of the other three *Dionysia* species by chromatographic comparison and by its colour properties.

Primetin (5,8-dihydroxyflavone) was identified in the farina of *P. chionantha* (cf. Ref. 4) by direct comparison with both a natural specimen supplied by Professor Hattori and a synthetic specimen supplied by Professor W. Baker. Material from all three sources had the following properties: λ_{max} in EtOH 282, 366, in EtOH–$AlCl_3$, 296, 360, and in EtOH–NaOEt, 290, 350 nm; R_f 0·76 on SiO_2 in 10% HOAc in $CHCl_3$, 0·68 on SiO_2 in 45% EtOAc in C_6H_6, 0·91 on paper in *n*-BuOH–HOAc–H_2O (4:1:5) and 0·24 in 15% HOAc; yellow in visible light, dull brown in u.v. light, immediate blue with Folin Ciocalteu reagent. Primetin differed in R_f, colour and/or spectral maxima from a number of other simple flavones examined at the same time, including 5-, 6-, and 7-monohydroxyflavone, 5,6-, 7,8- and 3,4′-dihydroxyflavone and 5-hydroxy-8-methoxyflavone.

Acknowledgements—The author thanks Professor P. Wendelbo for his generous interest and advice and, with Mr. Archibald, for the provision of the plant material. He also thanks Professor W. Baker for kindly supplying specimens of natural and synthetic flavones.

[5] W. NAGAI and S. HATTORI, *Acta Phytochim., Japan* **5**, 1 (1930).
[6] W. BAKER, N. C. BROWN, and J. A. SCOTT, *J. Chem. Soc.* 1922 (1939).

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PROTEACEAE

METHYL (*p*-HYDROXYBENZOYL) ACETATE AND AN ALKALOID, BELLENDINE, FROM *BELLENDENA MONTANA*

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Abstract—The previously unreported methyl (*p*-hydroxybenzoyl) acetate, and the first alkaloids from the Proteaceae, have been isolated from the flowers of *Bellendena montana*. The major alkaloidal constituent, bellendine, was obtained crystalline and characterized spectroscopically.