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FLAVONE AND FLAVONOL 5-GLUCOSIDES*

C. W. GLENNIE and J. B. HARBORNE
Phytochemical Unit, Department of Botany, The University, Reading, Berks.

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Abstract—Kaempferol and isorhamnetin 5-glucosides have been identified for the first time in plants in flowers and leaves of *Cotula* species (Compositae) A number of new sources of related 5-glucosides have been detected. Thus, quercetin 5-glucoside, previously reported in *Lamium* (Labiatae), has been identified in *Anthemis cotula* and in seven other taxa in the tribe Anthemideae-Compositae. Luteolin 5-glucoside has been found in *Cotula turbinata* and in *Leycesteria formosa* (Caprifoliaceae) Tricin 5-glucoside, previously found in *Triticum* (Gramineae) has been detected in two *Carex* species (Cyperaceae) A study of these 5-glucosides has shown that they are readily distinguished from more commonly occurring glucosides by R_f, colour and spectral properties and by their instability. The lability of the 5-*O*-glucoside link is indicated by the fact that flavone and flavonol 5-glucosides are hydrolysed by acid sixty times more rapidly than the corresponding 7-glucosides

INTRODUCTION

FLAVONOID 5-glycosides are interesting taxonomic markers in higher plants both because of their relative rarity and their ease of detection. In a previous paper,[1] the properties and systematic distribution of luteolin 5-glucoside were described and the provisional identification of the first flavonol 5-glucoside, that of quercetin, was reported. In the course of continuing chemotaxonomic studies in families of the Sympetalae and particularly in the Compositae,[2] a number of new sources of 5-glycosides has been uncovered. As a result, it has been possible to confirm the identification of quercetin 5-glucoside, in addition, two new 5-glucosides, those of kaempferol and isorhamnetin, have been discovered. These results are reported in the present paper.

RESULTS

In the course of a two-dimensional paper chromatographic survey of members of the Anthemideae–Compositae,[2] a number of plants were found to have yellow fluorescent flavonoid constituents. Because of the very intense fluorescence, it was very easy to score plants for their presence/absence and thus these compounds appeared to be convenient markers worthy of further study. The only known flavonoids with comparable fluorescent properties were azaleatin (quercetin 5-methyl ether) and related flavonol 5-methyl ethers[3] and a compound provisionally identified as quercetin 5-glucoside in *Lamium album* petals.[1] Since none of the fluorescent compounds was present in acid hydrolysed extracts of the composite plants, the possibility of their being 5-methyl ethers was ruled out and all these novel compounds therefore appeared to be flavonol 5-glycosides. This was confirmed by detailed studies of the more prominent fluorescent constituents.

* Part XV in the series 'Comparative Biochemistry of the Flavonoids'

[1] J B HARBORNE, *Phytochem* **6**, 1569 (1967)
[2] J B HARBORNE, V H HEYWOOD and N A M SALEH, *Phytochem.* **9**, 2011 (1970)
[3] J B HARBORNE, *Phytochem* **8**, 419 (1969)

The major fluorescent constituent in leaves of *Anthemis cotula* was isolated and characterized as quercetin 5-glucoside on the basis of its R_f, colour properties, spectral characteristics and hydrolytic products (Table 1) It was also identical in every way with the compound previously detected in *Lamium*.[1] Similar studies of several *Cotula* species revealed the presence of two new flavonol 5-glucosides, those of kaempferol and isorhamnetin, and these were characterized in the same way as the quercetin derivative.

The location of the sugar on the 5-hydroxyl in these flavonols was based on the close similarity in fluorescence and spectral properties (Table 1) with the analogous 5-methyl

Table 1. R_f and spectral characteristics of flavone and flavonol 5-glucosides

Glucoside*	BAW	R_f (×100) in 15% HOAc	H_2O	Phenol
Isorhamnetin 5-glucoside	38	08	01	68
Kaempferol 5-glucoside	26	34	10	45
Quercetin 5-glucoside	22	08	02	23
Quercetin 7-glucoside	23	08	02	28
Luteolin 5-glucoside	36	12	01	56
Tricin 5-glucoside	26	08	00	89

Glucoside	λ_{max} (nm) in EtOH Band I	Band II	Δλ (nm) in NaOAc Band I	$AlCl_3$ Band II	NaOEt Band III	H_3BO_3 Band II
Isorhamnetin 5-glucoside	255	370	2	55	77	0
Kaempferol 5-glucoside	263	359	11	60	49	0
Quercetin 5-glucoside	254	368	18	59	67	10
Quercetin 7-glucoside	257	275	0	50	46	10
Luteolin 5-glucoside	250 261†	345	14	19	56	23
Tricin 5-glucoside	265	348	—	0‡	72	0

* Monoglucosidic structures for the new flavonol 5-glucosides are advanced on the basis of R_f properties (compare similarity in R_f between quercetin 5- and 7-glucosides), of identifying glucose as the sole sugar of acid hydrolysis and of their ready β-glucosidase hydrolysis

† Inflection

‡ Incorrectly recorded as a positive shift in a previous publication,[6] the error presumably being due to the presence of free tricin in the 5-glucoside sample

Table 2 Rates of acid hydrolysis of flavonoid 5-glucosides

Glucoside	Time required for 50% hydrolysis
Isorhamnetin 5-glucoside	25 sec
Kaempferol 5-glucoside	25 sec
Quercetin 5-glucoside	35 sec
Luteolin 5-glucoside	10 sec
Tricin 5-glucoside	10 sec
Quercetin 7-glucoside	15 min
Luteolin 7-glucoside	20 min

TABLE 3. DISTRIBUTION OF FLAVONE AND FLAVONOL 5-GLUCOSIDES IN THE ANGIOSPERMS

Family, genus and species	5-Glucoside of*
Rosaceae	
Docyniopsis tschonoski Koidzumi (bark)	Chrysin[4]
Prunus serrata, P. speciosa (bark)	Genkwanin (apigenin 7-methyl ether)[4]
Leguminosae	
Amorpha fruticosa (leaf)	Apigenin[4]
Galega officinalis L (seed)	Luteolin[1,4]
Genista spp. (leaf)	Luteolin (see Note Added in Proof)
Umbelliferae	
Chaetosciadium trichospermum (L.) Boiss (leaf)	
Torilis arvensis (Hudson) Link (leaf)	Luteolin[1]
T. nodosa (L) Gaertner (leaf, seed)	
Labiatae	
Lamium album L (petal)	Quercetin[1]
Compositae†	
Anacyclus pyrethrum DC (leaf)	Quercetin
Anthemis cotula L (leaf)	Quercetin
Cotula australis Hook f (leaf)	Quercetin, isorhamnetin
C. barbata DC (leaf)	
C goughensis Rudmose Brown (leaf, petal)	Kaempferol
C turbinata (leaf)	Quercetin, luteolin
Dendranthema arcticum (petal)	Quercetin
Caprifoliaceae	
Leycesteria formosa Wall (leaf, bract, petal, stem)	Luteolin[5]
Gramineae	
Triticum dicoccum, T. monococcum	
T polonicum	
Oryza sativa	Tricin[6,7]
Cyperaceae	
Carex acutiformis Ehrhart	
C. riparia Curtis	

* References to the literature are given where appropriate, entries without reference numbers are new reports

† Flavonol 5-glycosides have also been provisionally detected in the following taxa: *Chrysanthemum coronarium* (leaf), *C segetum* (petal), *Coleostephus myconis* (leaf/petal), *Leucanthemum atratum* (leaf/petal), *L silvaticum* (leaf), *L vulgare* (leaf) and *Tripleurospermum inodorum* (leaf/petal)

ethers. However, the most significant indication that this linkage was present in these compounds was their very rapid rate of hydrolysis. These are shown, together with those of the similarly constituted flavone 5-glucosides in Table 2. It can be seen that, on average, the rate of hydrolysis of a 5-glucoside is 60 times faster than that of the corresponding 7-glucoside. This lability is obvious during isolation procedures, since these compounds continually break down during chromatography and handling. Another characteristic feature is their ability to absorb strongly onto cellulose and it proved very difficult to elute them from chromatography paper. Both these factors prevented the isolation of these compounds in sufficient quantity for NMR or i.r. spectral measurements.

The known distribution of flavonol and flavone 5-glucosides is outlined in Table 3, and this includes results of earlier studies[4] as well as our own surveys. It is clear that, within the tribe Anthemideae of the Compositae, these derivatives are widespread and occur in both subtribes. The only genus so far apparently lacking them is *Tanacetum* (absent from 4 species). A new record outside the Compositae is of luteolin 5-glucoside in *Leycesteria formosa*, which represents the only occurrence of this type of compound in the Caprifoliaceae after a survey of 57 species.[5] Another new record is of tricin 5-glucoside, previously known in *Triticum* (Gramineae),[6] in *Carex* (Cyperaceae); the taxonomic significance of this finding will be discussed elsewhere.[7]

The distribution of 5-glucosides within the plant has also been studied and is of some interest. In general, these compounds seem to be restricted to one or at the most two different parts of the plant, mostly frequently leaf and/or flower in the case of the Compositae. In *Galega officinalis*, luteolin 5-glucoside was only detected in the seed and could not be traced in any other tissue. Again, in *Torilis*, the same compound was generally restricted to the leaf; however, in *T. nodosa*, it was also noted in the seed. Exceptionally in *Leycesteria*, luteolin 5-glucoside was found throughout the plant (flowers, bracts, leaves, stem) apart from the roots.

DISCUSSION

Present surveys show that the 5-glucoside character occurs sporadically in eight angiosperm families (Table 3) most of which are to some extent inter-related. Thus, two of them, the Rosaceae and the Leguminosae, are generally accepted as belonging to the same order, the Rosales. Again, the present discovery of 5-glucosides in the Compositae is not surprising in view of the many other links, particularly in flavonoid chemistry[8,9] between the Leguminosae and the Compositae. Further, the Compositae and the Caprifoliaceae, with a record in *Leycesteria*, are usually placed in adjacent order by most systematists. Finally, the occurrence of 5-glucosides in the Umbelliferae and Compositae is of interest, since these are both highly specialized families which are already chemically linked through the presence of polyacetylenes in both groups.[10] One can conclude, therefore, that the presence of 5-glucosides is apparently restricted to phylogenetically advanced plants and this character shows promise of being a useful systematic marker in several plant groups.

While the function of 5-*O*-glucosylation of flavones and flavonols in plants is not known, it is apparently similar to that of 5-*O*-methylation in the sense that it blocks the normal 5-hydroxyl-4-carbonyl chelating system and at the same time makes the flavone or flavonol intensely fluorescent and thus possibly an attractant, at least when present in flowers, to insect pollinators. The distribution pattern of the 5-methyl ethers, however, is very different from that of the 5-glucosides since the former are restricted to six relatively primitive, mainly

[4] S HATTORI, in *Chemistry of the Flavonoid Compounds* (edited by J. A GEISSMAN), pp 316–352, Pergamon Press, Oxford (1962)
[5] C W. GLENNIE and B A BOHM, in preparation
[6] J B HARBORNE and E HALL, *Phytochem.* **3**, 421 (1964)
[7] J B HARBORNE, in preparation.
[8] E C. BATE-SMITH, *J. Linn Soc Botan* **58**, 39 (1962).
[9] J B HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967)
[10] N A SØRENSEN, in *Chemical Plant Taxonomy* (edited by T SWAIN), pp 219–252, Academic Press, London (1962)

woody dicotyledonous families.[3] The development within the angiosperms of the 5-*O*-methylation process therefore must have preceded 5-*O*-glucosylation as a means of producing fluorescent flavonoids in plant leaves and flowers.

EXPERIMENTAL

Plant Material

The majority of plants of the Compositae were grown from botanic garden seed at this University and voucher specimens have been deposited in the Department of Botany herbarium Material of *Cotula goughensis* was kindly provided by Dr. D. M. Moore from his collection of Gough Island and Tierre del Fuego plants growing at the University Other plants studied were collected locally.

Flavonoid Identifications

Flavonoids were extracted, separated, purified and identified by standard procedures.[9] Rates of acid hydrolysis of the various glucosides were measured as described earlier,[11] except that chromatograms were run on thin layers of cellulose and the relative amounts of aglycone and glucoside present after a given time interval were estimated by visual inspection in u v light

Note Added in Proof. In collaboration with Dr. Dale Smith, we have recently identified the 5-glucosides of quercetin and patuletin in leaves of the Polemoniaceae, in *Linanthus* (5 spp) and in *Linanthastrum nuttallii*.[12]

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[11] J B. Harborne, *Phytochem.* 4, 107 (1965).
[12] J B Harborne, *Phytochem* 8, 1449 (1969)