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CHEMOSYSTEMATICS OF THE COMPOSITAE: FLAVONOID PATTERNS IN THE *CHRYSANTHEMUM* COMPLEX OF THE TRIBE ANTHEMIDEAE

J. B. HARBORNE, V. H. HEYWOOD and N. A. M. SALEH*

Phytochemical Unit, Department of Botany, The University, Reading

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Abstract—Petal and leaf flavonoids have been identified in twenty-one species from seven genera of the Compositae-Anthemideae. A number of novel pigments have been found, including the 7-glucoside of quercetagetin 3′-methyl ether, which occurs in flowers of *Chrysanthemum coronarium*. 7-Glucuronides of apigenin, luteolin and chrysoeriol have been detected in the tribe for the first time; these three flavones also occur as a range of 7-diglycosides and chrysoeriol as an acylated glycoside. The distribution of these flavonoids supports the views of some systematists that the *Chrysanthemum* complex should be separated into a number of genera rather than maintained as a single genus. Thus, the species of *Chrysanthemum* (*sensu stricto*) studied are characterized by the presence of quercetagetin, patuletin and quercetin 7-glucosides, whereas those of *Anthemis* have only patuletin 7-glucoside. By contrast, *Leucanthemum* species have apigenin 7-glucuronide and *Tripleurospermum* luteolin 7-glucoside. Finally, *Tanacetum* species are characterized by the presence of luteolin and chrysoeriol 7-glucuronides and quercetin 7-glucoside.

INTRODUCTION

WHILE a considerable number of taxonomically interesting low molecular weight constituents have been reported in the Compositae (for summary see Hegnauer),[1] their distributions have not been widely studied and chemical data have not yet been applied on any scale to the solution of specific taxonomic problems in the family. Particularly "difficult" are the taxa within the tribe Anthemideae and the present investigation was undertaken to see if chemical data could hep in the classification of these plants.

The tribe Anthemideae, with about sixty genera, is often divided into two subtribes, the Anthemidinae and Chrysantheminae, largely on the basis of presence or absence of scales on the receptacle, although this division is recognized to be artificial. Relationships within the tribe are far from clear, although it is possible to discern clusters of species which have many morphological and anatomical characters in common. Most attention has been paid to the *Chrysanthemum* complex in which there are two main treatments: that of Hoffmann[2] who adopted a very wide circumscription of *Chrysanthemum* so as to include *Leucanthemum*, *Pyrethrum* and *Tanacetum*; and that of Briquet,[3] who maintained these and other genera as separate, largely as a result of his carpological studies.

Briquet's treatment has been reinforced by later taxonomic work,[4-8] although cytological data have not contributed much because of the uniformity of the base number ($x = 9$).

* Present address: Department of Botany, University of British Columbia, Vancouver, Canada.

[1] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. III, pp, 447–544, Birkhauser-Verlag, Basel (1964).
[2] O. HOFFMANN, *Natürl. Pflfam.* **4** (5), 267 (1894).
[3] J. BRIQUET and F. CAVALLIER, in *Flore des Alpes Maritimes* (edited by E. BURNAT), Vol. 6, pp. 1–344, Lyon (1916–17).
[4] M. GIROUX, *Bull. Soc. Hist. Nat. Afr. Nord.* **24**, 54 (1933).
[5] G. HARLING, *Act. Hort. Berg.* **16**, 1 (1951).
[6] V. H. HEYWOOD, *Anal. Inst. Bot. A. J. Cavanill.* **12**, 313 (1954).
[7] V. H. HEYWOOD, *Proc. Bot. Soc. British Isles* **3**, 177 (1959).
[8] V. H. HEYWOOD, *Agron. Lusitana* **20** (3), 205 (1959).

Recent phytochemical studies have also tended to support the recognition of several genera within the *Chrysanthemum* complex. Thus, several distribution patterns of polyacetylenes can be discerned within the group,[9] and the presence or absence of anthocyanin pigment in the root separates *Leucanthemum* from *Tanacetum* and *Chrysanthemum* proper.[10] A range of other chemical compounds occurs within the group and the present study was initiated to see if flavonoid data in general fitted in with the Briquet classification.

A number of flavonoid glycosides have been detected variously in plants of the Anthemideae (see Refs. 1, 11) although no systematic survey of the leaves and flowers has yet been undertaken. Luteolin and apigenin 7-glucoside are known to be widespread, while acacetin 7-rutinoside is reported from *C. morifolium* and tansy, *T. vulgare*. Quercetin 7-glucoside occurs in a number of species, including corn-marigold *C. segetum*, the flowers of which contain the yellow flavonol gossypetin 7-glucoside.[12] The flavonoid aglycones in the leaves of many Anthemideae have been surveyed,[13] and aglycones found additional to those mentioned above were kaempferol, isorhamnetin and patuletin. The isomeric 3′ and 4′-methyl ethers of luteolin, namely chrysoeriol and diosmetin, have also recently been recorded in *T. vulgare* by Khvorost.[14] Our present studies have shown the presence of a further aglycone, quercetagetin 3′-methyl ether, not previously known in any other plant, and of a number of new glycosides of the aforementioned aglycones.

RESULTS

The flavonoids in flower parts and leaves of twenty-one species of the *Chrysanthemum* complex were separately isolated and identified and the results are summarized in Table 1. The presence/absence of each component was carefully checked by two-dimensional chromatography of the direct plant extracts, and, because of the complex mixtures of flavonoids in some taxa, three different solvent–pair systems were employed.

Of the four flavonol glycosides reported, two have been found previously in the Anthemideae. Thus, quercetin 7-glucoside (quercimeritrin) has been isolated from *C. segetum* and *C. coronarium* by Steelink and his co-workers.[12,15] It has now been found in three further *Chrysanthemum* species, in *Dendranthema arcticum* and in *Tanacetum* (four spp.). It was not, however, detected in *Matricaria chamomilla* flowers, as reported by Hörhammer *et al.*;[16] this is not surprising, however, in view of the known variability of plant source of the "chamomile" flowers that are used in pharmacy. The other known flavonol, quercetagetin 7-glucoside, was found in *C. coronarium* flowers by Anyos and Steelink[15] and has now been detected in flowers of three further *Chrysanthemum* species (Table 1). Its presence in *C. segetum* is of special interest, since the petals of this plant also contain gossypetin 7-glucoside, a fact first recorded by Geissman and Steelink[12] and confirmed by us. This is the first and only record in the Anthemideae of the co-occurrence of the 6- and 8-hydroxylated derivatives of quercetin, i.e. quercetagetin and gossypetin, in the same plant. This finding is all the more remarkable since general surveys of plants for yellow flavonols have indicated that the two

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[10] C. FAVARGER, *Rev. Cyt. Biol. Vég.* **29**, 191 (1966).
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[12] T. A. GEISSMAN and C. STEELINK, *J. Org. Chem.* **22**, 946 (1957).
[13] H. GREGER, *Naturwiss.* **56**, 467 (1969).
[14] P. P. KHVOROST, *Chem. Abs.* **71**, 19517m (1969).
[15] T. ANYOS and C. STEELINK, *Arch. Biochem. Biophys.* **90**, 63 (1960).
[16] L. HÖRHAMMER, H. WAGNER and B. SALFER, *Arzneimittell. Forsch.* **13**, 33 (1963).

TABLE 1. DISTRIBUTION OF FLAVONE AND FLAVONOL GLYCOSIDES IN PLANTS OF THE ANTHEMIDEAE

Plant species	Parts examined‡	Flavones*							Flavonols*		
		1	2	3	4	5	6	7	1	2	3
Anthemis carpatica Waldst. & Kit.	Ray and disc	−	−	−	−	−	−	−	−	+	−
A. nigrescens Willd.	Ray and disc	−	+	−	+	−	−	−	−	+	−
A. tinctoria L.	Ray and disc	−	−	−	−	−	−	−	−	+	−
Chrysanthemum carinatum Schousbeo§	Leaf	−	−	−	−	−	−	−	+	+	−
C. coronarium L.	Leaf, ray and disc	−	−	+	+	−	+	−	+	−	+
C. nivellei Maire	Leaf, disc	−	−	−	−	−	−	−	+	+	+
C. segetum L.	Leaf, disc and ray	−	−	+	−	−	−	−	+	−	+
C. viscidohirtum (Schott) Thellung	Leaf, ray	−	−	−	−	−	−	−	+	+	+
Dendranthema arcticum (L.) Tzvelev	Ray and disc	−	+	−	−	−	−	−	+	+	−
Leucanthemum silvaticum (Hoffm. & Link) Nyman	Flower	−	+	−	−	−	−	−	−	−	−
L. atratum (Jacq.) DC.	Ray and disc	−	+	−	−	−	−	−	−	−	−
L. vulgare Lam.	Leaf and flower	−	+	−	−	−	−	−	−	−	−
Matricaria chamomilla L.	Leaf and ray	+	−	+	−	+	−	−	−	−	−
Tripleurospermum phaeocephalum (Rupr.) Pobed.	Flower	+	−	−	−	+	−	−	−	−	−
T. inodorum (L.) Schultz Bip.	Leaf and disc	−	−	+	−	−	−	−	−	−	−
T. maritimum (L.) Koch	Leaf, ray and disc	+	−	+	−	−	−	−	−	−	−
T. tchichatchevii (Boiss.) Bornm.	Leaf	−	−	+	−	−	−	−	−	−	−
Tanacetum coccineum (Willd.)	Ray and disc	−	−	−	+	−	−	+	+	−	−
T. corymbosum (L.) Schultz Bip	Ray and disc	−	−	−	+	−	−	+	+	−	−
T. macrophyllum (Waldst. & Kit.) Schultz Bip.	Flower	−	−	−	+	−	−	+	+	−	−
T. niveum (Lag.) Schultz Bip.	Ray and disc	−	+	−	+	−	−	+	+	−	−

* Key—Flavones: 1, apigenin 7-glucoside; 2, apigenin 7-glucuronide; 3, luteolin 7-glucoside; 4, luteolin 7-glucuronide; 5, luteolin 7-rutinoside; 7-glucoside; 7, chrysoeriol 7-glucuronide. Flavonols: 1, quercetin 7-glucoside; 2, patuletin 7-glucoside; 3, quercetagetin 7-glucoside.

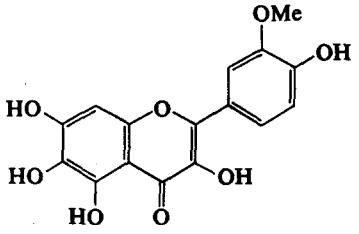
† Key—A, quercetagetin 3′-methyl ether 7-glucoside and chrysoeriol 7-rutinoside (in flower); B, gossypetin 7-glucoside (in ray and disc); C, a glucuronide; D, apigenin 7-glucoside isomer (in ray); E, cyanidin 3-glucoside (in ray); F, chrysoeriol 7-(*p*-coumaroylglucosylglucuronide); and xylosylglucuronide.

‡ Since not all plant parts were consistently available for analysis, no distinction is made in this table in flavonoid distribution within the pla flavones 1–7 and flavonol 1 were present in both leaf and flower, while flavonols 2, 3 were only in the flower.

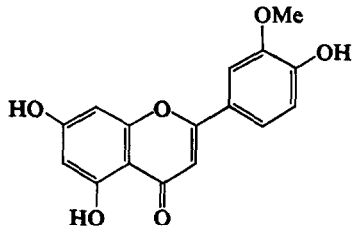
§ Fourteen named varieties of late summer-flowering *Chrysanthemum* uniformly contained luteolin 7-glucoside and apigenin 7-glucuronide in flower. Garden forms of *C. coronarium* and *C. segetum* had additional pigments to those present in the wild collections.

classes of pigment differ considerably in their distribution, gossypetin being confined generally to the less specialized higher plants with quercetagetin characterizing the more advanced taxa.[17]

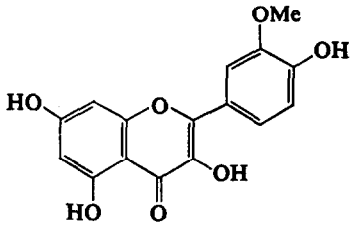
Patuletin or quercetagetin 6-methyl ether, previously known as an aglycone in the Anthemideae,[13] has now been identified as the 7-glucoside in *Anthemis*, *Chrysanthemum* and *Dendranthema* (see Table 1). The fourth flavonol glycoside, detected only in petals of *coronarium*, is the 7-glucoside of a new flavonoid aglycone, namely the 3′-methyl ether of



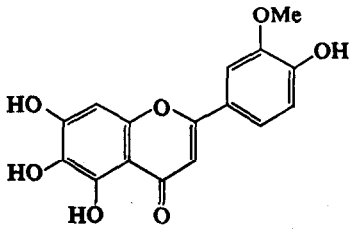
Quercetagetin 3′-methyl ether (I)



Chrysoeriol (II)



Isorhamnetin (III)



(IV)

quercetagetin (I). It was identified by standard microscale procedures. Its mass spectrum and its ready demethylation to quercetagetin indicated it was a quercetagetin monomethyl ether. U.v. spectral analysis indicated that substitution was in the 3′- or 4′-position, since the spectrum gave no borate shift. Further, spectral measurements on the 7-glucoside in alkaline solution showed that it contained a free 3,4′-dihydroxyl group. The presence of a 3′-methoxyl group was finally confirmed by reductive cleavage, which gave 3-methoxy-4-hydroxyphenylpropionic acid, and not the isomeric 3-hydroxy-4-methoxy derivative.

The discovery of quercetagetin 3′-methyl ether in the Anthemideae is of biogenetic interest, in view of the presence of the corresponding flavone, chrysoeriol (II), in many of these plants (see below) and of the corresponding quercetin derivative, namely isorhamnetin (III), although this particular compound was not found during the present work. The recent report[18] of the closely related 5,6,7,4′-tetrahydroxy-3′-methoxyflavone (IV) (trivial name: batatifolin or nodifloretin)[19] in *Mikania batatifolia* (tribe Eupatorieae, Compositae) is also relevant to the present discovery.

Two of the three flavones found in the Anthemideae species studied, luteolin and apigenin, are well known as composite flavones.[11] By contrast, the third, chrysoeriol, is relatively rare and has only been previously found in the Compositae in *T. vulgare*.[14] It has now been dis-

[17] J. B. HARBORNE, in *Recent Advances in Phytochemistry* (edited by J. WATKIN), Vol. IV, Appleton-Century-Crofts, New York, in press.

[18] W. HERZ, P. S. SANTHANAN, H. WAGNER, R. HOER, L. HÖRHAMMER and L. FARKAS, *Tetrahedron Letters* 3419 (1969).

[19] A. K. BARVA, P. CHAKRABARTIS and P. K. SANYAL, *J. Ind. Chem. Soc.* **46**, 271 (1969).

covered in *C. coronarium*, where significantly it occurs in association with the related quercetagetin 3′-methyl ether, and in the four *Tanacetum* species studied (see Table 1).

Each of the three Anthemideae flavones occurs both as the common 7-glucoside and as the relatively uncommon 7-glucuronide. Indeed, 7-glucuronides have not previously been reported in the Anthemideae and do not appear either to have been recorded before in the Compositae. As a group, the three flavone glucuronides seem to be of considerable systematic interest, since they occur characteristically in *Leucanthemum* and *Tanacetum* and are generally absent from *Chrysanthemum* (except *C. coronarium*), *Tripleurospermum* and *Matricaria* (see Table 1). Apigenin, luteolin and chrysoeriol also occur in a number of other glycosidic forms, all of which are of more limited distribution and some species specific. They are the 7-rutinosides of luteolin and chrysoeriol (in *M. chamomilla*, *T. phaeocephalum* and in *C. coronarium*), the 7-acylglucuronide of apigenin (in *Dendranthema*), a 7-xylosylglucuronide of apigenin (in *Tanacetum niveum*) and a complex *p*-coumaroylglucosylglucuronide of chrysoeriol (in *Tanacetum corymbosum*). Finally, there is evidence in *Matricaria* of an apigenin 7-glucoside which is isomeric with the common 7-β-D-glucoside and differs presumably in its sugar linkage; its structure is being further studied.

DISCUSSION

The present limited survey of taxa of the Anthemideae has indicated that the flavonoid pattern within the tribe is complex. The results of this and earlier work show the presence in the *Chrysanthemum* complex of no less than twelve flavonoid aglycones, each capable of existing in one or more of six glycosidic combinations. The heterogeneity of flavonoid patterns within the group confirms the systematic view that *Chrysanthemum* complex are best separated into several genera and thus reinforce the data* obtained from polyacetylene studies.[9]

If one accepts the Briquet classification[3] as modified by Heywood,[6–8] one finds that flavonoid characters are very useful for delineating taxa at the generic level (see Table 1). Thus, species of *Chrysanthemum* (*sensu stricto*) are characterized by the presence of the 7-glucosides of quercetin, quercetagetin and patuletin, whereas *Anthemis* species have only the latter pigment. By contrast, *Leucanthemum* (three species studied) has a very simple pattern, with apigenin 7-glucuronide being the sole flavonoid present in leaf and flower. *Tripleurospermum* (four species studied), which is often included in *Matricaria*, is distinguished by the presence of luteolin 7-glucoside. However, *M. chamomilla*, the only *Matricaria* studied, is very similar in its flavonoid pattern. *Tanacetum* (four species studied) has three characters in common; luteolin, chrysoeriol and quercetin 7-glucosides.

The results of these flavonoid analyses can also be interpreted in evolutionary terms, if one accepts the view that flavones are advanced over flavonols and that *O*-methylation and 6- or 8-hydroxylation represent gain mutations.[11,17] Thus, the taxa can be classified according to whether they have flavonols only (two *Anthemis*, three *Chrysanthemum*), both flavonols and flavones (one *Anthemis*, two *Chrysanthemum*, *Dendranthema* and all *Tanacetum*), or flavones alone (*Leucanthemum*, *Matricaria*, *Tripleurospermum*). One may also note that 3′-*O*-methylation is restricted to *C. coronarium* and *Tanacetum* and that 6- or 8-hydroxylation only occurs in the flavonols of *Anthemis*, *Chrysanthemum* and *Dendranthema*. These suggest

* In the paper of Bohlmann *et al.*[9] the classification of Hoffmann is used; in addition, the nomenclature requires reinterpretation.

groupings of the various taxa which are not very different from the generic classification and which may possibly also express their phylogenetic relationships.

In view of the limited sampling of the genera, the above results will doubtless require modification when further species have been examined. Furthermore, only the most prominent flavonoids have been identified in the present study and a number of other pigments, including those located in the cytoplasmic fraction such as the lipid soluble quercetagetin 3,6,7,3′-tetramethyl ether of *M. chamomilla*,[20] and also related phenolics (e.g. hydroxycoumarins) still require survey. In addition, variation in flavonoid pattern almost certainly exists at the species level and future studies will be concerned with extending the survey to natural populations of those species with wide geographical distribution.

EXPERIMENTAL

Plant Material

The majority of plants were grown from botanic garden seed and, in general, voucher specimens have been deposited in the Reading University herbarium. Plants of *Chrysanthemum segetum* were collected on Ladle Hill, near Kingsclere, Hampshire, while those of *Matricaria chamomilla*, *Tripleurospermum maritimum* and *Leucanthemum vulgare* were collected locally in Reading, Berkshire. Garden forms of *C. carinatum* and other *Chrysanthemum* cultivars were obtained from the Botanic Garden of the Department of Horticulture of this University.

Flavonoid Identifications

Two-dimensional paper chromatographic surveys of leaf, disc and ray extracts of the plants were carried out using the solvent pairs: *n*-BuOH–HOAc–H_2O (4:1:5) (BAW) and 15% HOAc; BAW and PhOH–H_2O: and BAW and *n*-BuOH–EtOH–H_2O (4:1:2·2). R_f values for all the components, run one-dimensionally by descent on Whatman No. 1 paper, are given in Table 2. Known flavonoids were identified by standard procedures (see e.g. Ref. 11) and in most cases compared directly with authentic samples.

Quercetagetin 3′-Methyl Ether 7-Glucoside

This was isolated from petals of *C. coronarium* and had λ_{max} in EtOH at 260, 279 and 364 nm; the spectrum was unaffected by addition of NaOAc or NaOAc–H_3BO_3, but gave a bathochromic shift with $AlCl_3$ ($\Delta\lambda + 24$ nm). In the presence of alkali, there was a shift in the long-wave band to 410 nm, but this peak decomposed fairly rapidly (30 per cent reduction in absorbance in 4 min) and at approximately the same rate as kaempferol (35 per cent reduction in 4 min). Since the A-ring pyrogallol system in this flavonoid is stabilized by glucosylation in the 7-position, the instability of the alkaline spectrum must be due to the presence of a free 3,4′-dihydroxyl group.[21]

Acid hydrolysis gave glucose and quercetagetin 3′-methyl ether as a yellow powder. Its λ_{max} in EtOH were at 257, 277 and 366 nm; the spectrum was unaffected by addition of NaOAc–H_3BO_3, but gave a bathochromic shift with $AlCl_3$ ($\Delta\lambda + 31$ nm) and with alkali ($\Delta\lambda + 42$ nm; rapid decomposition). The mass spectrum gave a parent ion peak at 332 ($C_{16}H_{12}O_8$ requires mol. wt. 332) and there was only slight fragmentation at 317 ($-CH_2$) (*ca.* 5% of parent ion peak), indicating the absence of methylation at the 6-hydroxyl group (cf. Ref. 22); other minor peaks were at 303, 289 and 274. Demethylation with pyridinium chloride at 140° for 3 hr gave quercetagetin. Reductive cleavage gave 3-methoxy-4-hydroxyphenylpropionic acid, which was separated on silica gel plates two-dimensionally, using the standard system,[23] from the isomeric 3-hydroxy-methoxyphenylpropionic acid by the fact that it gave a positive Folin reaction and a pink colour with Gibbs reaction (the isomer gave a blue colour).

[20] R. HÄNSEL, H. RIMPLER and K. WALTHER, *Naturwiss.* **53**, 19 (1966).
[21] L. JURD, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 126, Pergamon Press, Oxford (1962).
[22] J. H. BOWIE and D. W. CAMERON, *Australian J. Chem.* **19**, 1627 (1966).
[23] H. M. HURST and J. B. HARBORNE, *Phytochem.* **6**, 1111 (1967).

TABLE 2. R_f VALUES AND COLOUR REACTIONS OF FLAVONE AND FLAVONOL GLYCOSIDES OF THE ANTHEMIDEAE

Flavonoid	BAW	PhOH	15% HOAc	Colour in u.v. light without and with NH_3‡
Apigenin 7-glucoside	68	81	31	Dull brown → bright green
Apigenin 7-glucoside*	77	93	31	
Apigenin 7-glucuronide	52	52	21	
Apigenin 7-acylglucuronide	69	68	37	
Apigenin 7-xylosylglucuronide	26	16	42	
Luteolin 7-glucoside	38	63	15	Dull brown → bright green
Luteolin 7-glucuronide	40	29	18	
Luteolin 7-rhamnosylglucoside	35	63	34	
Chrysoeriol 7-glucoside	44	84	32	Dull brown → very bright yellow-green
Chrysoeriol 7-glucuronide	45	48	17	
Chrysoeriol 7-rhamnosylglucoside	35	78	32	
Chrysoeriol 7 (*p*-coumaroylglucosylglucuronide).	48	48	38	
Quercetin 7-glucoside	29	31	09	Bright yellow
Quercetagetin 7-glucoside	17	14	05	Very dark brown
Quercetagetin 3′-methyl ether 7-glucoside	23	54	05	
Gossypetin 7-glucoside	23	29	07	
Patuletin 7-glucoside	35	49	12	Bright yellow
Quercetagetin 3′-methyl ether	55	58	43†	Very dark brown
Quercetagetin	38	13	28†	

* Isomeric 7-glucoside in *Matricaria chamomilla*.
† R_fs in Forestal.
‡ While flavones show colour changes with NH_3, flavonols do not.

New Flavone Glycosides

An *apigenin 7-glucoside* was isolated from the rays of *M. chamomilla* which, after purification, had the same R_fs in aqueous solvents, colour reactions and spectral properties as an authentic sample of apigenin 7-β-D-glucoside, but differed in R_f in BAW and PhOH (Table 2). Insufficient material was available for further characterization, but it appeared to be an isomer, in which the sugar linkage was α-, instead of β-, or else where the sugar was in the furanose rather than the pyranose form. Apigenin 7-xylosylglucuronide (from *Tanacetum niveum* flower) and apigenin 7-acylglucuronide (from *Dendranthema arcticum* flower) behaved in every respect like other apigenin 7-diglycosides and gave the appropriate sugars and aglycone on acid hydrolysis; the latter also gave apigenin 7-glucuronide as an intermediate of alkali or enzyme hydrolysis, but was present in insufficient amount for the identification of its presumably aliphatic acyl group.

Chrysoeriol 7-rhamnosylglucoside, isolated from *C. coronarium* leaf, was identical in spectral properties to other chrysoeriol 7-glycosides and gave glucose, rhamnose and chrysoeriol (in approximate equal amounts) on hydrolysis. The chrysoeriol 7-(*p-coumaroylglucosylglucuronide*) from *T. corymbosum* had λ_{max} 254, 272, 321 with an inflection at 350 nm, indicating the presence of an acyl substituent. Alkaline hydrolysis gave *p*-coumaric acid and acid hydrolysis gave glucose, glucuronic acid and chrysoeriol. β-Glucuronidase hydrolysis gave chrysoeriol 7-glucuronide as an intermediate.

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