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Variations in lipophilic and polar flavonoids in the genus *Tanacetum*

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Abstract

Seven species of *Tanacetum* of different geographical origins were analysed in leaf and flower for their lipophilic and polar flavonoids and the results were compared with the patterns previously recorded in *T. parthenium* and *T. vulgare*. The lipophilic constituents are based generally on 6-hydroxykaempferol 3,6,4′-trimethyl ether and quercetagetin 3,6,3′-trimethyl ether, but up to 11 other surface flavonoids are present. Methyl ethers of scutellarein and 6-hydroxyluteolin are present in species with corymbose capitula. A rare carbomethoxyflavone reported earlier in *T. microphyllum* could not be detected. The dominant pattern of vacuolar flavonoids is based on apigenin and luteolin 7-glucuronides. 6-Hydroxyluteolin 7-glucoside, present in *T. vulgare*, also occurs in *T. pseudoachillea*. Chrysoeriol 7-glucuronide is present in *T. parthenium*, *T. macrophyllum* and *T. corymbosum*. By contrast, quercetin 7-glucuronide characterises *T. parthenium*, *T. corymbosum* and *T. cinerariifolium*. On the basis of combined data of lipophilic and polar constituents, it is clear that several structures are useful chemotaxonomic characters at the species level in a taxonomically complex genus.

Keywords: *Tanacetum*; Compositae; Flavonoids; Chemotaxonomy

1. Introduction

A recent reinvestigation of the flavonoids of feverfew, *Tanacetum parthenium* (L.) Sch. Bip., and tansy, *T. vulgare* L., revealed a rich mixture of lipophilic and polar constituents variously present in leaf, ray-floret and disc-floret (Williams, Harborne, Geiger & Hoult, 1999a). The major surface flavonoids were tested pharmacologically and shown to have useful anti-inflammatory properties (Hoult, Pang, Bland-Ward, Forder, Williams & Harborne, 1995; Williams et al., 1999a). These reinvestigations, based on PC, TLC and HPLC, revealed more than twice the number of structures previously recorded in feverfew and tansy. It, therefore, seemed appropriate to extend the reinvestigation to seven further *Tanacetum* species, with the hope of finding new *O*-methylated flavones and flavonols for pharmacological evaluation.

One of the seven species chosen for further analysis was *T. balsamita* L., which comes from Europe and Central Asia, and is grown as the aromatic herb, costmary. *T. microphyllum* DC. is endemic to Spain and Portugal, while *T. cilicium* (Boiss.) Grierson is of Turkish origin. The fourth species, *T. corymbosum* (L.) Sch. Bip, is widespread in Europe and yields an antibacterial oil. Two further species, *T. macrophyllum* (Waldst. and Kit.) Sch. Bip. and *T. cinerariifolium* (Trev.) Sch. Bip, originate from the Balkans but are cultivated elsewhere, especially *T. cinerariifolium* which is grown for its insecticidal pyrethrins. The seventh species, *T. pseudoachillea* Winkl., was of Russian origin. A second accession of *T. vulgare* L., which was available in European botanic gardens under the incorrect labelling of *T. huronensis* Nutt., was also analysed.

Some earlier work has been reported on the flavonoids of the above species. Thus, luteolin and chry-

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Table 1
The distribution of surface flavonoids in *Tanacetum* species

Species	Part of Plant	Surface flavonoids[a]											
		Flavonols				Flavones							
		1	2	3	4	5	6	7	8	9	10	11	12
T. parthenium (L.) Schultz Bip.	L[b]	+	+++	+	++	(+)	–	–	–	–	–	–	–
	D	(+)	+++	+	++	(+)	–	–	–	–	–	–	–
	R	(+)	+++	++	+++	+	++	(+)	–	–	–	–	–
T. microphyllum DC.	L	+	+++	+	++	–	–	–	–	–	–	–	–
	F[c]	–	++	+	++	–	++	+	–	–	–	–	–
T. vulgare L.	L	–	–	(+)	–	–	–	–	+	–	(+)	++	++
	D[d]	–	–	–	+	++	++	++	–	–	–	–	–
T. vulgare L. variant	L[b]	–	–	(+)	–	–	–	–	–	+	–	++	++
	D[d]	–	–	–	+	+	++	+	–	–	–	–	++
T. cilicium (Boiss.) Grierson	L	–	+++	–	++	(+)	–	–	(+)	+	–	+	–
	F	–	+++	–	++	+	++	++	–	++	–	+	–
T. macrophyllum (Waldst. and Kit.) Schultz Bip.	L	–	+++	–	++	(+)	–	–	(+)	+	–	+	–
	F	–	+++	–	++	+	++	+	–	++	–	+	–
T. cinerariifolium (Trev.) Schultz Bip.	L	++	–	+	+++	(+)	–	–	++	(+)	–	–	–
	D	–	–	–	+++	(+)	–	–	+	–	–	–	–
	R	–	–	–	+++	–	–	–	+	+	–	–	–
T. corymbosum (L.) Schultz Bip.	L	–	–	–	–	–	–	–	–	–	–	–	–
	D	–	–	(+)	–	–	–	–	(+)	–	+	–	–
	R	–	–	–	–	–	–	–	(+)	–	+	–	–
T. pseudoachillea Winkler	L	–	–	++	–	–	–	–	++	–	–	–	–
	F	–	–	++	–	–	–	–	+	–	++	–	–
T. balsamita L.	L	–	–	–	–	(+)	–	–	++	–	++	++	++

[a] Key: (1) 6-hydroxykaempferol 3,6-dimethyl ether; (2) 6-hydroxykaempferol 3,6,4′-trimethyl ether; (3) quercetagetin 3,6-dimethyl ether; (4) quercetagetin 3,6,3′-trimethyl ether (accompanied by isomeric 3,6,4′-trimethyl ether); (5) apigenin; (6) luteolon; (7) chrysoeriol; (8) scutellarein 6-methyl ether; (9) scutellarein 6,4′-dimethyl ether; (10) 6-hydroxyluteolin 6-methyl ether; (11) 6-hydroxyluteolin 6,3′-dimethyl ether; (12) 6-hydroxyluteolin 6,7,4′-trimethyl ether. L = leaf, D = disc floret, R = ray floret, F = flower head.

[b] The sesquiterpene lactone, parthenolide was also present.

[c] Kaempferol 3,4′-dimethyl ether, quercetin 3-methyl ether and 3,4′-dimethyl ether were also present in trace amount.

[d] Quercetagetin 3,6,3′,4′-tetramethyl ether was also present.

soeriol 7-glucuronides, with quercetin 7-glucoside, were found in flowers of *T. corymbosum* and *T. macrophyllum* (Harborne, Heywood & Saleh, 1970). An investigation of *T. cinerariifolium* leaf and flowerhead gave the 7-glucosides and 7-glucuronides of apigenin, luteolin and quercetin, together with the 3,6-dimethyl ether and 3,6,4′-trimethyl ether of quercetagetin (Glennie & Harborne, 1971). Finally, four anti-inflammatory flavonoids were recorded in *T. microphyllum*. One of these four flavonoids, as reported, is unique among known structures in having a carboxymethyl substituent at C-7 and is 5,3′-dihydroxy-3,4′-dimethoxy-7-carboxymethyl flavone (Abad, Bermejo, Villar & Valverde, 1993; Martinez, Silvan, Abad, Bermejo & Villar, 1997).

Taxonomically, the genus *Tanacetum* L. with 150 species, is recognised to be difficult to classify (Bremer & Humphries, 1993). This is reflected in the fact that cladistic analysis of morphological characters has shown it to be paraphyletic (Bremer, 1994). Chemical data on the genus is generally sparse (Greger, 1977), so that further investigation of flavonoids in the genus is potentially useful taxonomically. The present paper records the lipophilic and polar flavonoids found in the above seven *Tanacetum* species.

2. Results

The results of analysing *Tanacetum* species for their lipophilic flavonoids are shown in Table 1 and for their polar flavonoids in Table 2. Two accessions of *T. vulgare* were examined, with similar but not identical results. Other accessions of several species were examined (results not shown) and were generally found to be identical in flavonoid profile with the given accession. In particular, a collection of *Tanacetum* species growing in the Chelsea Physic Garden was compared with the collection grown from seed at Reading and found to be generally identical. In view of the difficulties in ascertaining the correct names for plants of Botanic Garden origin, all the taxa that were examined in detail were verified by a taxonomic expert.

The lipophilic flavonoids present in leaf, ray or disc

Table 2
The distribution of vacuolar flavonoids in *Tanacetum*

Species	Part of plant	Apigenin glycosides					Luteolin glycosides				Other glycosides[a]			
		1	2	3	4	5	6	7	8	9	10	11	12	13
T. parthenium (L.) Schultz Bip.	L	–	+	–	–	–	+	+	–	–	+	–	–	–
	D	–	+	–	–	–	+	+	–	–	–	–	+	–
	R	–	+	+	+	–	–	–	–	–	–	–	–	–
T. microphyllum DC.	L	–	–	–	–	–	+	+	–	–	–	–	–	–
	F	–	–	–	–	–	+	+	–	+	–	+	–	–
T. vulgare L.	L	+	+	–	–	–	+	+	–	–	–	–	–	–
	D	–	–	–	–	–	+	+	–	+	–	+	–	–
T. vulgare L. variant	L	+	+	–	–	–	+	+	+	–	–	+	–	–
	D	–	+	–	–	–	+	+	–	+	–	+	–	–
T. cilicium (Boiss.) Grierson	L	–	+	–	+	–	+	+	–	–	–	–	–	–
	F	–	+	–	–	–	+	+	–	–	–	–	–	–
T. macrophyllum (Waldst. and Kit.) Schultz Bip.	L	–	+	–	–	–	+	–	–	–	–	–	–	–
	F	–	–	–	–	–	–	+	–	–	+	–	–	–
T. cinerariifolium (Trev.) Schultz Bip.	L	+	+	–	–	–	+	+	–	–	–	–	–	–
	D	+	+	–	–	+	+	+	–	–	–	–	+	–
	R	–	+	–	–	+	–	–	–	–	–	–	–	–
T. corymbosum (L.) Schultz Bip.	L	–	–	–	–	–	+	+	–	–	–	–	–	–
	D	–	–	–	–	–	–	+	–	–	+	–	–	+
	R	–	–	–	–	–	–	+	–	–	+	–	–	+
T. pseudoachillea Winkler	[b]L	–	–	–	–	–	+	–	–	+	–	–	–	–
	F	+	–	–	–	–	–	–	–	–	–	–	–	–
T. balsamita L.	L	–	+	–	–	–	–	+	–	–	+	–	–	–

[a] Key: (1) apigenin 7-glucoside; (2) apigenin 7-glucuronide; (3) apigenin 7-glucosylglucuronide; (4) apigenin 7-diglucuronide; (5) schaftoside; (6) luteolin 7-glucoside; (7) luteolin 7-glucuronide; (8) luteolin 7-gentiobioside; (9) 6-hydroxyluteolin 7-glucoside; (10) chrysoeriol 7-glucuronide; (11) diosmetin 7-glucuronide; (12) quercetin 7-glucuronide; (13) quercetin 7-glucoside. L = leaf, D = disc floret, R = ray floret, F = flower head.
[b] This species also contains chrysoeriol 7-glucoside and a luteolin 7-diglucoside.

of *Tanacetum* species fall into two biosynthetic series. There are the methylated flavonols formed by progressive *O*-methylation of quercetagetin and 6-hydroxykaempferol, as illustrated in Fig. 1. Then, there are the methylated flavones formed by *O*-methylation of 6-hydroxyluteolin and scutellarein, as illustrated in Fig. 2. In *T. parthenium*, quercetagetin 3,6,3′-trimethyl ether was accompanied by the isomeric 3,6,4′-trimethyl ether in leaf, disc floret and ray floret. The same mixture of two isomers is possibly present generally in the genus, although the presence of the 3,6,4′-trimethyl ether was not verified in every case.

What is noticeable in the methylation patterns in the flavonol series (Fig. 1) is the apparent absence of 7-*O*-methylation. Such *O*-methylation is uncommon among flavonoids of the Compositae compared to the regular appearance of 6-*O*-methylation, but it has been detected in *Pulicaria dysenterica* (L.) Bernh., a plant included as an outgroup to *Tanacetum*, where three 7-*O*-methylated quercetagetin derivatives as well as 6-hydroxykaempferol 3,7,4′-trimethyl ether are present (Williams, Harborne & Greenham, 1999b; Wollenweber, Darr, Fritz & Valant-Vetschera, 1997).

In the flavone series of surface constituents (Table

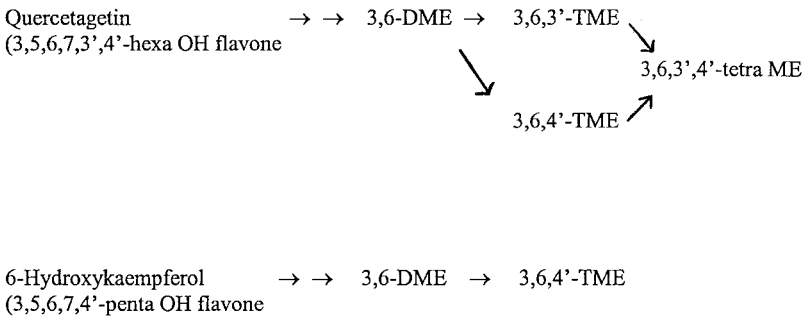

Fig. 1. Pathway of lipophilic flavonol methylation in *Tanacetum* (DME = dimethyl ether; TME = trimethyl ether; tetra ME = tetramethyl ether).

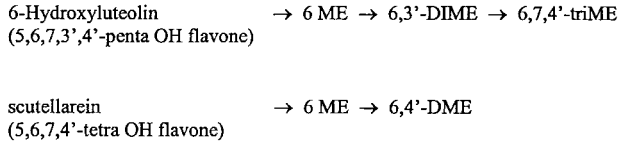

Fig. 2. Pathway of lipophilic flavone methylation in *Tanacetum*.

1), methylation of 6-hydroxyluteolin variously at the 6-, 3′-, and 4′-positions is dominant. Nevertheless, 7-*O*-methylation does occur, 6-hydroxyluteolin 6,7,4′-trimethyl ether being characteristically present in *T. vulgare*. While 6-hydroxyflavonols in methylated form occur in all species surveyed, the methylated 6-hydroxyflavones are present in six of the eight species, but do not appear in *T. parthenium* or *T. microphyllum*.

Earlier studies of the Spanish species *T. microphyllum* showed the presence of four lipophilic flavonoids: 6-hydroxykaempferol 3,6,4′-trimethyl ether, quercetagetin 3,6,4′-trimethyl ether, kaempferol 3,4′-dimethyl ether and the biogenetically unique 3,5,3′-trihydroxy-4′-methoxy-7-carboxymethylflavone (Martinez et al., 1997). In our work on this species, we were able to confirm the presence of the first three flavonoids. In addition, we detected six further lipophilic components in leaf or ray: quercetagetin 3,6-dimethyl ether, 6-hydroxykaempferol 3,6-dimethyl ether, quercetin 3-methyl ether, quercetin 3,4′-dimethyl ether, luteolin and chrysoeriol. These are all broadly expectable on biogenetic grounds or correspond to lipophilic flavonoids present elsewhere in *Tanacetum* (Table 1). However, we were unable to find any evidence for the presence of a flavonol with a carboxymethyl substituent at the 7-position and we seriously doubt the structural identification of this particular *T. microphyllum* constituent.

The lipophilic flavonoids of *T. balsamita* aerial parts have been previously examined by Wollenweber et al. (1997) and our results (Table 1) agree broadly with the earlier findings. It is interesting that two further species, *T. cilicium* and *T. macrophyllum* are identical in their lipophilic constituents (Table 1). Nevertheless, comparison of their respective polar flavonoid profiles (see Table 2) show at least five differences.

The lipophilic flavonoids recorded for nine European *Tanacetum* species can be compared with earlier results recorded for *T. polycephalum* Sch. Bip., an Iranian species, *T. chiliophyllum* C.A. Mey. and *T. santolinoides* (DC.) Feinbrun and Fertig. In the first two of these three species, the patterns (Wollenweber, Mann & Valant-Vetschera, 1989; Wollenweber & Rustaiyan, 1991) are broadly similar to those reported in Table 2. The third species *T. santolinoides* differs somewhat in having more highly methylated compounds, namely 6-hydroxyluteolin 6,7,3′,4′-tetramethyl ether and quercetagetin 3,6,7,3′,4′-pentamethyl ether (El-Din, El-Serbakhi & El-Ghazouly, 1985).

The polar, vacuolar flavonoids recorded in the various *Tanacetum* species are shown in Table 2. The major constituents are apigenin and luteolin 7-glucuronides, often accompanied by lesser amounts of the corresponding 7-glucosides. *Tanacetum pseudoachillea* is apparently unique in this sample in having only the 7-glucosides. Two series of luteolin 7-diglucosides are present in *Tanacetum*. Thus, luteolin 7-gentiobioside has been found in *T. microphyllum*. An isomeric luteolin 7-diglucoside was detected in *T. pseudoachillea*, but apart from the fact that it was chromatographically separable from the gentiobioside, it could not be further characterised because of lack of material.

The 7-glucuronides of two luteolin methyl ethers, chrysoeriol and diosmetin were found to occur occasionally. Chrysoeriol 7-glucuronide is present in four species (Table 2), while the corresponding 7-glucoside was detected in *T. pseudoachillea*. By contrast, diosmetin 7-glucuronide was confined to a single source, *T. vulgare*. One may also note that 6-hydroxyluteolin 7-glucoside, recently reported for the first time in *Tanacetum* in *T. vulgare* (Williams et al., 1999a), is also present in *T. pseudoachillea*.

Other classes of water-soluble flavonoid, besides the above flavones, are only occasionally reported in these plants (Table 2). One may note the presence of quercetin 7-glucuronide in *T. parthenium* and *T. cinerariifolium* and of quercetin 7-glucoside in *T. corymbosum*. These quercetin 7-glycosides are mainly found as constituents of the disc florets. Glycoflavones are uncommon; only one such derivative, schaftoside (apigenin 6-*C*-glucoside-8-*C*-arabinoside) was present and this only in *T. cinerariifolium*. Earlier investigations of *T. corymbosum* revealed the presence of an acylated flavone glycoside, namely chrysoeriol 7-(*p*-coumarylglucosylglucuronide) (Harborne et al., 1970). No other examples of acylated flavones were recorded during the present investigation. Finally, it may be noted that flavanones as a class were not evident in any of the *Tanacetum* species surveyed here. However, derivatives of naringenin and eriodictyol were earlier reported to occur in *T. sibiricum* (Stepanova, Scheichenko, Smirnova & Glyzin, 1981).

3. Discussion

Results of the present analysis of seven species of *Tanacetum*, combined with the earlier reinvestigation of two further species, have revealed a complex pattern of both surface and vacuolar flavonoids in leaf, ray-floret and disc-floret of these plants. Although earlier investigations have been carried out on the flavonoids of some of these plants, they have rarely revealed the

true complexity of flavonoid constituents. In all, some 17 lipophilic and 15 polar flavonoids were characterised (Tables 1 and 2). In the present work, attention has been concentrated on those substances that accumulate in these plants, and undoubtedly further investigations are likely to yield a variety of trace constituents as well.

From the chemotaxonomic viewpoint, there is no doubt that the flavonoid patterns are useful at the species level. Each species has a number of distinctive features, either in terms of presence/absence or of variation in the distribution in the different organs of the plant. The present investigations reveal once more the potential that flavonoid pigments have for illuminating taxonomic relationships (Harborne & Turner, 1984). In the case of *Tanacetum*, there are still many species which are poorly known (Bremer & Humphries, 1993; Bremer, 1994). Undoubtedly, flavonoid investigations would add significantly to the further classification of these particular plants.

Our present study of *Tanacetum* flavonoids was motivated by an interest in the anti-inflammatory activity of the lipophilic constituents and such activity was determined for the flavonols and flavones of *T. parthenium* and *T. vulgare* (Williams et al., 1999a, 1999b). It is clear from the relative uniform pattern of lipophilic constituents of all the *Tanacetum* species now surveyed (Table 1), that further interesting structures for pharmacological testing are uncommon. We are, therefore, currently reinvestigating the flavonoids of related genera in the same tribe, Anthemideae, as *Tanacetum* in the hope of uncovering further novel structures.

4. Experimental

4.1. Plant material

Fresh leaves, disc florets and, when available, ray florets were separately collected from plants grown at the School of Plant Sciences, the University of Reading from seed supplied by European botanical gardens as follows: *T. parthenium* from Nymphenberg, Germany; and *T. cilicium*, *T. cinerariifolium*, *T. corymbosum*, *T. macrophyllum*, *T. pseudoachillea* and two specimens of *T. vulgare* from the Botanic Garden, Berlin, Germany. Seed of *T. microphyllum* was kindly collected for us by a plant taxonomist from the Madrid herbarium, Spain. Voucher specimens were prepared of all plants studied and these were verified by Dr. Nicholas Hind of the Herbarium, Royal Botanic Gardens, Kew. One plant species, labelled *T. huronensis* and grown as such, turned out to be a form of *T. vulgare*. Since its analysis differed slightly from that of the normal tansy, *T. vulgare*, it has been included in Tables 1 and 2 as a variant of *T. vulgare*. It also differed in yielding the sesquiterpene lactone parthenolide, which is a characteristic terpenoid of *T. parthenium* but is not usually recorded for *T. vulgare* (Seaman, 1982). The collection of *Tanacetum* species growing at the Chelsea Physic Garden was kindly made available to us by the Director and the results compared with those obtained from the Reading botanic garden material.

4.2. Flavonoid analyses

The flavonoids were extracted separately from fresh leaf, disc floret and ray floret by dipping briefly in $Me_2CO \times 3$ for 30 s intervals. These extracts were then concentrated and separated by TLC by using the procedures outlined in an earlier paper (Williams et al., 1999a). Lipophilic flavonoids were purified and characterised by identical procedures described therein. Plant tissue, following Me_2CO washing, was extracted into boiling 80% MeOH and left to extract overnight. These extracts were separately concentrated and then chromatographed on sheets of Whatman No. 3 paper in *n*-BuOH–HOAc–H_2O (4 : 1 : 5, top layer). The flavonoid bands so obtained were eluted with 80% MeOH and rerun on no. 3 paper in 15% aqueous HOAc. At this stage most flavonoids were pure enough for analysis, but if not, they were further chromatographed until pure. General methods of analysis have been described elsewhere (Williams et al., 1999a). In practically all cases, co-chromatography in four solvents with authentic glycosides was combined with UV spectral analysis and hydrolysis to aglycone and sugar. The identity of glucuronides was always confirmed by their mobility during paper electrophoresis in NaOAc buffer pH 4.4 at 30 v/cm for 2 h. Luteolin 7-gentiobioside was identified in ray florets of *T. microphyllum* by its R_f values relative to luteolin 7-glucoside (in parenthesis): 0.28 (0.44) in BAW, 0.04 (0.15) in *n*-BuOH–EtOH–H_2O (4 : 1 : 2.2), 0.3 (0.2) in H_2O and 0.24 (0.15) in 15% HOAc. On acid hydrolysis, it gave luteolin, glucose and a disaccharide gentiobiose, confirmed by co-chromatography in three solvents with authentic material. Literature data and UV spectral analysis further supported this identification.

4.3. Mass spectral measurements

Lipophilic flavonoids: apigenin ex *T. cilicium* requires MW 270, EI-MS 270, 153 (A-ring) 121 (B-ring); scutellarein 6,4′-dimethyl ether ex *T. cilicium* requires MW 314, EI-MS 314, 299 (M-15), 271 (M-43), 153 (A-ring), 133 (B-ring); 6-hydroxykaempferol 3,6,4′-trimethyl ether ex *T. macrophyllum* requires MW 344, EI-MS 344, 329 (M-15), 301 (M-43) 135 (B-ring); quercetagetin 3,6,3′-trimethyl ether ex *T. macrophyllum* requires MW 360, EI-MS 360, 345 (M-15).

Water soluble flavonoids: schaftoside ex *T. cinerariifolium* requires MW 564, FAB-MS −ve mode 563, +ve mode 565, fragmentation at 298 corresponding to apigenin 8-aldehyde; apigenin 7-glucuronide from *T.* spp. requires 446, FAB-MS −ve mode 445, +ve mode 447, EI-MS 270.

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