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A BIOLOGICALLY ACTIVE LIPOPHILIC FLAVONOL FROM *TANACETUM PARTHENIUM*

CHRISTINE A. WILLIAMS, J. R. S. HOULT,* JEFFREY B. HARBORNE, JENNY GREENHAM and JOHN EAGLES†

Department of Botany, School of Plant Sciences, University of Reading, Whiteknights, Reading RG6 2AS, Berkshire, U.K.; *Pharmacology Group, King's College London, Manresa Road, London SW3 6LX, U.K.; †Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, U.K.

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Key Word Index—*Tanacetum parthenium*; Compositae; feverfew; leaf, flower and seed; lipophilic flavonoids; 6-hydroxyflavonol methyl ethers; 6-hydroxykaempferol 3,7,4′-trimethyl ether.

Abstract—A new lipophilic flavonol, 6-hydroxykaempferol 3,7,4′-trimethyl ether, called tanetin, has been characterized in the leaf, flower and seed of feverfew, *Tanacetum parthenium*. It co-occurs with the known 6-hydroxykaempferol 3,7-dimethyl ether, quercetagetin 3,7-dimethyl ether and quercetagetin 3,7,3′-trimethyl ether. Pharmacological tests indicate that tanetin could contribute to the anti-inflammatory properties of feverfew by inhibiting the generation of pro-inflammatory eicosanoids, although it is unlikely to be the only biologically active compound within the plant. Water soluble flavone glycosides were detected in the leaves and identified as apigenin 7-glucuronide, luteolin 7-glucuronide, luteolin 7-glucoside and chrysoeriol 7-glucuronide.

INTRODUCTION

Tanacetum parthenium (L.) Schultz Bip., a perennial herb commonly known as feverfew, has been grown since Roman times for its many medicinal properties. Both fresh leaf and commercially available dried leaf preparations are being currently used as a herbal remedy for the control of migraine and arthritis, following recent clinical trials that have confirmed its value in the treatment of migraine [1, 2]. The active principles have been suggested to be parthenolide and other sesquiterpene lactones containing an α-methylenebutyrolactone residue [3]. These compounds are capable of reacting covalently with sulphydryl groups in proteins [4], which could explain the time-dependent inhibitory effects of feverfew extracts on platelet and leukocyte secretion and be responsible for their anti-inflammatory and cytotoxic properties.

In identifying the active principles of this plant, it is important to be aware of the nature of the other chemical constituents since there is the possibility of synergistic action. Although the sesquiterpene lactones have been fully identified in feverfew, little work has been carried out on the lipophilic flavonoids of *T. parthenium*. However, such compounds are likely to be present since several flavonoid methyl ethers have been characterized from other *Tanacetum* species. Thus, 6-hydroxyluteolin 6,3′-dimethyl ether (jaceosidin) [5] and quercetagetin 3,6,3′-trimethyl ether (jaceidin) [5] have been reported from the flowers of *T. vulgare*, while 6-hydroxyluteolin 6,7,3′-trimethyl ether (cirsilineol), 6-hydroxyluteolin 6,7,3′,4′-tetramethyl ether [6] and quercetagetin 3,6,7,4′-tetramethyl ether (casticin) [6] have been found in aerial parts of *T. santolinoides*.

More recently, a unique flavonoid structure with a carboxylic acid substituent in the 7-position, 3,5,3′-trihydroxy-4′-methoxy-7-carbomethoxyflavone has been characterized from *T. microphyllum* together with quercetagetin 3,6,4′-trimethyl ether [7].

The present paper reports the lipophilic flavonoids identified from the leaf, flower and seed of *T. parthenium* and the flavonoid glycosides present in the leaf vacuoles.

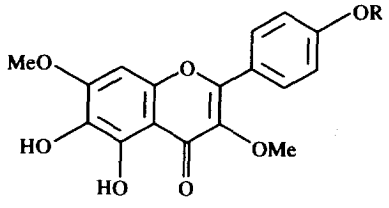
RESULTS AND DISCUSSION

In an HPLC diode array analysis of chloroform extracts of dried leaves, flowers and seed of *T. parthenium* four lipophilic flavonoids were detected in all three tissues. In the acetone washings of fresh feverfew leaves an additional minor flavonoid constituent was detected. Thus, five flavonoids (**1**–**5**) were isolated from the acetone leaf extract by silica gel TLC and purified by paper chromatography and Sephadex LH20 column chromatography. The major flavonoid component in leaf, flower and seed was **1**, which was characterized by means of R_f, HPLC, EI-MS and UV spectral data (Table 1 and Experimental) as 6-hydroxykaempferol 3,7,4′-trimethyl ether, a new flavonol methyl ether, which we name tanetin. Thus, the longwave band at 337 nm in the methanol UV spectrum of **1** suggested that it was a 3-substituted flavonol, and the single absorbance peak of

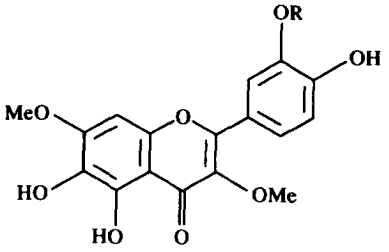
Table 1. R_f and HPLC data for flavonoid aglycones 1–5

6-Hydroxy-flavonol methyl ether	$R_f \times 100$ on cellulose in 30% HOAc	40% HOAc	50% HOAc	on silica gel in toluene–HOAc (4:1)	HPLC R_t*
1	56	82	93	54	13.10
2	36	51	70	08	5.75
3	52	68	82	19	7.42
4	49	68	82	30	7.84
5	23	40	68	09	6.43

*C_{18}-phenyl reverse phase column at 25° using a linear gradient of 40% A: 60% B→100% B over 20 min, flow rate 1 ml min^{-1}. A = 2% HOAc, B = MeOH–HOAc–H_2O, 18:1:1. UV detection at 260 and 350 nm.



R
1 Me
3 H



R
2 H
4 Me

this band and its dark to dark colour in UV light that there was a free 6-hydroxyl in the molecule (related 8-hydroxyflavonols have two peaks in this band [8]). The negative sodium acetate shift indicated that the 7-hydroxyl is substituted and the small borate shift the absence of a free 3′,4′-*ortho*dihydroxy system in the B-ring. Similarly, a positive $AlCl_3$ confirmed that the 5-position was free and the lack of a shift back with HCl confirmed the absence of two free adjacent hydroxyl groups in the B-ring. EI-MS data gave a molecular weight of 344, corresponding to a dihydroxy-trimethoxyflavone. A strong [M − 15] ion at 319 (50% intensity) showed ready demethylation typical of a 3-methoxyl and a [M − 43] ion at 301 mu typical of flavonols. Finally, a fragment ion at 135 mu corresponded to a 4′-methoxy B-ring. Thus, the proposed structure of **1** is confirmed as 6-hydroxykaempferol 3,7,4′-trimethyl ether. It has been previously synthesized by Horie *et al.* [9], but this is the first report from a natural source.

Since there is an earlier report in a review article [10] of santin (6-hydroxykaempferol 3,6,4′-trimethyl ether) occurring in feverfew, it seemed important to further establish that our compound **1** is in fact the 7-methyl derivative. This was accomplished by treatment of **1** with hot pyridinium chloride for 2 hr. The well-known resistance of the 7-*O*-methyl group to demethylation was exhibited and the reaction yielded two identifiable intermediates, the 7-methyl ether and the 7,4′-dimethyl ether, as well as 6-hydroxykaempferol (see Experimental).

Direct comparison of the UV spectral data of tanetin **1** with the isomer santin, isolated from *Dodonea* [11], showed that **1** differed in exhibiting a small but significant (+4 nm) spectral shift in the presence of borate ion. We hypothesize that this shift is due to a weak complexing of borate with the 5,6-dihydroxy system in the A-ring. Such complexing, which must be weak because of the degree of hydrogen bonding between the 5-hydroxyl and the 4-carbonyl, does not appear to have been recorded before, although borate is known to give positive shifts with flavonols which have a free 6,7- or 7,8-dihydroxy system in the A-ring [12].

In order to confirm our hypothesis, we measured the borate spectra for six other flavonols with either the 5,6-dihydroxy-7-methoxy or 5,7-dihydroxy-6-methoxy substitution in the A-ring (Table 2). In all cases, the former compounds gave shifts, whereas the latter were unchanged. In the case of 6-hydroxykaempferol, which gives the largest shift (+15 nm), it is likely that the 6,7-dihydroxy chelate system is the major contributor to the recorded shift.

Three of the remaining flavonoids were identified as the known structures: quercetagetin 3,7-dimethyl ether (**2**), 6-hydroxykaempferol 3,7-dimethyl ether (**3**) and quercetagetin 3,7,3′-trimethyl ether (**4**) by standard procedures (R_f, HPLC, EI-MS and UV spectral data compared with authentic markers or lit. values). The fifth compound, a minor constituent, remains to be characterized.

Table 2. Borate spectra of 5,6-dihydroxy and 5,7-dihydroxyflavonols lacking a catechol B-ring

Flavonol	λ_{max} (nm) MeOH	MeOH–H_3BO_3	$\Delta\lambda$
5,6-Dihydroxy-7-methoxy			
Tanetin (**1**)	273, 337	341	4
Quercetagetin 3,7,3′-trimethyl ether	258, 271, 351	355	4
6-Hydroxykaempferol 3,7-dimethyl ether (**3**)	271, 341	347	6
6-Hydroxykaempferol 7-methyl ether	280, 350	362	12
6-Hydroxykaempferol	273, 350	365	15
5,7-Dihydroxy-6-methoxy			
Santin	274, 340	340	0
6-Hydroxykaempferol 3,6-dimethyl ether	276, 350	349	0
Quercetagetin 6,3′-dimethyl ether	259, 270, 373	371	0

These results show that the lipophilic flavonols of feverfew, **1**–**4**, are based on 6-hydroxykaempferol and quercetagetin, with *O*-methylation variously in the 3-, 7-, 3′- and 4′-positions. While 6-hydroxylation of flavonols and flavones is a common feature in *Tanacetum* species [5–7], the methylation pattern is distinctive for *T. parthenium*. Although **1** is unique in its occurrence in feverfew, the other three flavonols are known to occur elsewhere in the same family, the Compositae. Thus, **3** is reported in *Heterotheca inuloides*, while **2** is known from *Holocarpha obconica* and **4** (chrysosplenol C) from *Chrysosplenium alternifolium* [13].

Four commonly occurring flavone glycosides: apigenin 7-glucuronide, luteolin 7-glucuronide, luteolin 7-glucoside and chrysoeriol 7-glucuronide, were identified from an 80% methanolic leaf extract, made after removal of the surface lipophilic flavonoids.

Tanetin was tested as an inhibitor of eicosanoid generation, using rat peritoneal leukocytes activated by the calcium ionophore A 23187, and was found to inhibit both cyclo-oxygenase and 5-lipoxygenase pathways of arachidonate metabolism, with similar high potency. For example, at 40 μM tanetin inhibited the generation of leukotriene B_4, thromboxane B_2 and prostaglandin E_2 by 86.0 ± 2.7%, 97.0 ± 0.3% and 85.3 ± 2.2%, respectively (mean ± s.e.m., triplicate tests). Full details of these and other pharmacological tests on the lipophilic flavonols reported here in feverfew, will be published elsewhere [14]. Inhibition of eicosanoid generation by tanetin may contribute to the anti-inflammatory properties of feverfew, although it is unlikely to be the only biologically active compound within the plant [15].

EXPERIMENTAL

Plant material. Fr. leaf material was collected from plants grown at the School of Plant Sciences, University of Reading from seed obtained from Nymphenberg, Germany. A voucher specimen has been deposited in the University of Reading Herbarium (RNG) Dried leaf and flower material were obtained from plants grown at Alvechurch, Worcs and a further dried leaf sample and seeds from plants grown at Castle Donington, Leics, voucher specimens for which have been deposited in the Herbarium of the Department of Pharmacy, King's College, London.

Isolation of flavonoids. The flavonoids were extracted from fr. leaf material by dipping the leaves briefly in $Me_2CO \times 3$. Powdered dried leaves, flowers and ground seed were extracted by stirring with $CHCl_3$ for 1 hr. Compound **1** was isolated from the concd Me_2CO fr. leaf extract by multiple silica gel TLC in toluene–HOAc, 4:1. In this solvent **1** had a similar mobility to the sesquiterpene lactone, parthenolide (R_fs 56 and 54, respectively). The mixt. was eluted with MeOH and resolved by running the concd eluate on PPC in 30% HOAc. Compound **1** was further purified by Sephadex LH20 CC by washing first with H_2O and then elution with MeOH.

Compounds **2**–**5** were isolated by the same procedure from both the $CHCl_3$ dried flower extract and acetone leaf extract.

HPLC analysis of T. parthenium *tissue extracts.* HPLC diode array analysis of the Me_2CO fr. leaf and $CHCl_3$ dried leaf, flower and seed extracts was carried out on a Waters 994 instrument with a C_{18}-phenyl reverse phase column at 25° using a linear gradient of 40% A–60% B to 100% B over 20 min at a flow rate of 1 ml min^{-1}. A = 2% HOAc, B = MeOH–HOAc–H_2O, 18:1:1 with UV detection at 260 and 350 nm.

6-Hydroxykaempferol 3,7,4′-trimethyl ether (**1**). HPLC data: Table 1. EI-MS *m/z* 344, 319 [M − 15], 301 [M − 43] and 135 mu. UV λ_{max}^{MeOH} 273, 337; + NaOAc 274, 298, 372; + H_3BO_3 273, 341; + NaOH 274, 374 (low intensity); + $AlCl_3$ 280, 355, 390; and + $AlCl_3$–HCl 282, 354. High resolution mass measurement gave a measured mass 344.0894, $C_{18}H_{16}O_7$ requires 344.0896.

Demethylation of tanetin. Compound **1** was treated with pyridinium chloride at 120° for 2 hr under N_2. The reaction product was chromatographed on Whatman No. 3 paper in 40% HOAc and the bands cut out, eluted and their spectra determined. The main product was 6-hydroxykaempferol 7-methyl ether: UV λ_{max}^{MeOH} 280, 350; + NaOAc 280, 368; + NaOH 280, 390; + H_3BO_3 280, 362; + $AlCl_3$ 290, 400; + $AlCl_3$–HCl, 380 sh 428 nm. There was an equal amount of 6-hydroxykaempferol: UV λ_{max}^{MeOH} 273, 350; + NaOAc, 276, 372; + NaOH, 292, 396

(decomp. with time); $+H_3BO_3$ 282, 365; $+AlCl_3$ 283, 398 nm. A 3rd minor product was identified as the 7,4'-dimethyl ether: UV λ_{max}^{MeOH} 270, 360 nm from its R_f value and R_t on HPLC under standard conditions of 10.9 min.

Quercetagetin 3,7-dimethyl ether (**2**). R_f and HPLC data: Table 1. EI-MS *m/z* 346, 331 ([M−15] at 50% intensity of the molecular ion) and 137 mu (B-ring, 3',4'-DiOH). UV λ_{max}^{MeOH} 266, 359; +NaOAc 271, 398; $+H_3BO_3$ 266, 379; +NaOH 274, 325, 404; $+AlCl_3$ 274, 417 and $+AlCl_3$–HCl 264, 363.

6-Hydroxykaempferol 3,7-dimethyl ether (**3**). R_f and HPLC data: Table 1. EI-MS *m/z* 330, 315 [M−15], 287 ([M−43] flavonol fragment) and 121 mu (4'-OH B-ring). UV λ_{max}^{MeOH} 271, 341; +NaOAc 274, 376; $+H_3BO_3$ 274, 347; +NaOH 274, 325, 399; $+AlCl_3$ 274, 357 and $+AlCl_3$–HCl 278, 356. On co-HPLC with the isomeric 3,6-dimethyl ether, which had R_t 5.75 min, it showed a R_t of 7.71 min.

Quercetagetin 3,7,3'-trimethyl ether (**4**). R_f and HPLC data: Table 1. EI-MS *m/z* 360 and 345 [M−15] mu. UV λ_{max}^{MeOH} 258, 271, 351; +NaOAc 274, 390; $+H_3BO_3$ 272, 355; +NaOH 274, 340, 412; $+AlCl_3$ 266, 371; and $+AlCl_3$–HCl 264, 366.

Apigenin, luteolin and chrysoeriol 7-glucuronides and luteolin 7-glucoside were identified by standard procedures: R_f and UV data, acid hydrolysis to aglycone and sugar, and comparison with authentic markers.

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