



The flavonoids of *Tanacetum parthenium* and *T. vulgare* and their anti-inflammatory properties

Christine A. Williams^{a,*}, Jeffrey B. Harborne^a, Hans Geiger^b, J. Robin S. Hoult^c

^aDepartment of Botany, The University of Reading, Reading RG6 6AS, UK

^bFachrichtung 13.1 Botanik, Universität des Saarlandes, Postfach 1150, W-66041 Saarbrücken, Germany

^cPharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London SW3 6LX, UK

Received 24 November 1998; accepted 11 January 1999

Abstract

The lipophilic flavonoids in leaf and flower of *Tanacetum parthenium* and *T. vulgare* have been compared. While those of *T. parthenium* are methyl ethers of the flavonols 6-hydroxykaempferol and quercetagenin, the surface flavonoids of *T. vulgare* are methyl ethers of the flavones scutellarein and 6-hydroxyluteolin. Apigenin and two flavone glucuronides are surprisingly present in glandular trichomes on the lower epidermis of the ray florets of *T. parthenium*. The opportunity has been taken to revise the structures of the four 6-hydroxyflavonol methyl ethers of *T. parthenium* based on NMR measurements. These are now shown to be uniformly 6- rather than 7-*O*-methylated. Tanetin, previously thought to be a new structure, is now formulated as the known 6-hydroxykaempferol 3,6,4'-trimethyl ether. The vacuolar flavonoids of both plants are dominated by the presence of apigenin and luteolin 7-glucuronides; nine other glycosides were present, including the uncommon 6-hydroxyluteolin 7-glucoside in *T. vulgare*. When the major flavonol and flavone methyl ethers of the two plants were tested pharmacologically, they variously inhibited the major pathways of arachidonate metabolism in leukocytes. There were significant differences in potency, with the tansy 6-hydroxyflavones less active than the feverfew 6-hydroxyflavonols as inhibitors of cyclo-oxygenase and 5-lipoxygenase. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Tanacetum parthenium*; *T. vulgare*; Compositae; Feverfew; Tansy; 6-Hydroxykaempferol 3,6,4'-trimethyl ether; 6-Hydroxyluteolin 6,7,4'-trimethyl ether; Thromboxane B₂; Leukotriene B₄; Anti-inflammatory activity

1. Introduction

As part of an investigation of the biological properties of flavonoids in the genus *Tanacetum*, we reported earlier the discovery of four 6-hydroxyflavonol methyl ethers in the surface extracts of leaf, flower and seed of feverfew, *Tanacetum parthenium* (L.) Schultz Bip. (Williams, Hoult, Harborne, Greenham, & Eagles, 1995). The anti-inflammatory activity of the major flavonoid, called tanetin, was found to be significant (Hoult et al., 1995), particularly because feverfew is

currently used in the treatment of arthritis and migraine (Newell, Anderson, & Phillipson, 1996).

While the structures of the four flavonol methyl ethers of feverfew seemed to be securely based on EI-MS and UV spectral data, insufficient material was then available for confirmation by NMR. We have now isolated and separated larger amounts of these lipophilic constituents and here report the NMR results. These clearly show that in the earlier structures, the position of the A-ring methoxyl was incorrectly assigned to the 7- instead of the 6-position and that all four compounds are the respective 6-*O*-methyl ethers. At the same time, six other minor lipophilic flavonoids have been identified in feverfew tissues.

We also describe in this paper a parallel investigation of lipophilic flavonoids in the related composite

* Corresponding author. Tel.: +44-1734-318-168; fax: +44-7134-753-676.

plant, the tansy *Tanacetum vulgare* L. and report on their anti-inflammatory activities. Tansy is a well known medicinal plant, much used in the past, but is now contraindicated for deworming children because of the toxic monoterpene, α -thujone, present throughout the tissues (Newell et al., 1996). Previous investigations of tansy flowers have revealed the presence of quercetagenin 3,6,3'-trimethyl ether and 6-hydroxykaempferol 6,3'-dimethyl ether (Ognyanov, & Tochorova, 1983). A water-soluble apigenin 7-diglycoside has been found in the leaf of tansy (Khvorost, 1969). In this paper, we therefore record a comprehensive reinvestigation of lipophilic and water-soluble flavonoids in feverfew and tansy and describe some pharmacological properties of the main lipophilic constituents.

2. Results

2.1. Structural revision of the major lipophilic flavonols of feverfew

A comparison of brief acetone washings of leaf, flower and seed of feverfew indicated that the seed contained a very similar profile of flavonoids as the leaf. The dried seeds were used to isolate sufficient material for NMR analyses. Thus, the seed washings were purified on polyamide and Sephadex to give three flavonol methyl ethers in a combined yield of 0.1%. These were identical in all respects (R_f , R_t , UV spectra and shifts) to the same compounds previously obtained from leaf washings (Williams et al., 1995). They were identified as 6-hydroxykaempferol 3,6-dimethyl ether (**1**), 6-hydroxykaempferol 3,6,4'-trimethyl ether (**2**) and quercetagenin 3,6-dimethyl ether (**3**) respectively. The NMR data of **2** and **3** were within experimental error identical to published values (Barbera, 1986). Although NMR data for **1** measured in DMSO- d_6 were not available, its structure could be deduced by

comparing the NMR signals with those of **2** (Table 1). The differences between the two spectra are precisely those predicted by applying the rules in earlier reviews of flavonoid 1H and ^{13}C NMR data (Markham, & Chari, 1982; Markham, & Geiger, 1994).

The crucial evidence in the NMR data Table 1 is the absence of signals for a 7-methoxyl (^{13}C NMR signal predicted around 56.0 ppm) and the concomitant presence of signals at 59.0 to 60.0, which are due to 3- and 6-methoxyl substituents. Our original identification of these three flavonols as the isomeric 7-*O*-methyl ethers was therefore at fault. Hence, the main lipophilic constituents of feverfew are 6-hydroxykaempferol 3,6-dimethyl ether (**1**), santin (**2**) and axillararin (**3**). The claim that tanetin (6-hydroxykaempferol 3,7,4'-trimethyl ether) is a new natural plant substance (Williams et al., 1995) has thus to be withdrawn.

Two further lipophilic flavonols occur in minor amounts, together with **1**–**3**. Their presence was established when a sample of **3**, prepared for NMR measurements revealed a small percentage of two quercetagenin trimethyl ethers, **4** and **5**. The structures of **4** and **5** could not be established beyond doubt but the available data Table 1 fit best with the assumption that they are quercetagenin 3,6,3'-trimethyl ether (**4**) and quercetagenin 3,6,4'-trimethyl ether (**5**). Methylation of the 7-hydroxyl group can be excluded in both cases (c.f. Markham, & Geiger, 1994). Compound **4** was also obtained pure by preparative paper chromatography and measurement of its spectral and other properties were in agreement with its formulation as quercetagenin 3,6,3'-trimethyl ether.

2.2. Identification of flavonoids in surface extracts of different tissues of feverfew and tansy

Further separate examination of brief (50 s) acetone washings of leaf, disc and ray of feverfew plants revealed the presence of three constituents not so far

Table 1

NMR data of feverfew flavonols **1**–**5**. ^{13}C values are derived from the C-projection of a proton detected H–C correlation spectrum (500 MHz, DMSO- d_6 , ambient temperature) s = singlet, d = doublet, obsc = obscured by signals of **2**, signals of **4** and **5** assigned by 'best fit'

Flavonoid position	1		2		3		4		5	
	1H	^{13}C	1H	^{13}C	1H	^{13}C	1H	^{13}C	1C	^{13}C
8	6.53s	94.0	6.54s	94.0	6.50s	94.0	6.58	94.0	6.503	94.0
2'	7.91d ^o	130.0	7.99d ^o	130.0	7.52d ^{mm}	115.0	7.62d ^{mm}	112.0	7.52	115.0
3'	6.93d ^o	115.5	7.10d ^o	114.0	–	–	–	–	–	–
5'	6.93d ^o	115.5	7.10d ^o	114.0	6.89d ^o	116.0	6.92d ^o	116.0	7.08d ^o	112.0
6'	7.91d ^o	130.0	7.99d ^o	130.0	7.43dd ^{om}	120.5	7.55dd ^{om}	122.0	7.52dd ^{om}	120.0
(3 or 6)-OMe	3.73s	60.0	3.73s	60.0	3.73	60.0	obsc		obsc	
(3 or 6)-OMe	3.76s	59.0	3.76s	59.5	3.76	59.5	obsc		obsc	
3'-OMe	–	–	–	–	–	–	obsc		–	
4'-OMe	–	–	3.83s	55.0	–	–	–		obs	

Table 2
Lipophilic flavonoids of feverfew and tansy

	Feverfew			Tansy	
	leaf ^a	disc	ray	leaf	disc
<i>Flavonols</i> ^b					
6-Hydroxykaempferol					
3,6-Dimethyl ether (1)	+	(+)	–	–	–
3,6,4'-Trimethyl ether (2)	+++	+++	++	–	–
Quercetagenin					
3,6-Dimethyl ether (3)	+	++	–	++	–
3,6,3'-Trimethyl ether (4)	++	+++	+	–	+
3,6,4'-Trimethyl ether (5)	++	+++	+	–	–
3,6,3',4'-Tetramethyl ether	–	–	–	–	+
<i>Flavones</i>					
Apigenin	(+)	+	+	–	+++
Luteolin	–	++	–	–	+++
Chrysoeriol	–	(+)	–	–	+++
Scutellarein 6-methyl ether (6)	–	–	–	++	–
6-Hydroxyluteolin					
6-Methyl ether (7)	–	–	–	+	–
6,3'-Dimethyl ether (8)	–	–	–	+++	–
6,7,4'-Trimethyl ether (9)	–	–	–	+++	–
Apigenin 7-glucuronide	–	–	+	–	–
Luteolin 7-glucuronide	–	–	+	–	–

^a Feverfew seed contains the same compounds as the leaf in slightly different concentrations.

^b Amounts present: + + +, major; + +, medium; +, minor; (+), trace.

recognised in the free state in feverfew: apigenin, luteolin and chrysoeriol (Table 2). Additionally, washings of the ray flowers yielded two flavone glycosides, apigenin and luteolin 7-glucuronides. Although these two glycosides are readily detectable in 80% methanolic extracts of the rays, it was unexpected to find them also in the lipophilic extracts.

It is possible that the two glycosides were liberated by the acetone washings from internal cells, but if so, this is peculiar to feverfew, since similar experiments with several other *Tanacetum* species (Williams, unpublished experiments) failed to yield any glycosidic material in surface extracts. It may also be mentioned that Wollenweber, Stüber, and Kraut (1997) detected several flavonol glycosides, along with aglycones, in leaf exudates of *Nothofagus antarctica*.

Comparison of lipophilic constituents present on the surface of leaf, ray and disc indicates both quantitative and qualitative differences between the tissues (Table 2). All these compounds are presumably located in surface glands and indeed both the leaf and seed are known to have glandular hairs (Blakeman, & Atkinson, 1979). The same is true of ray florets, since scanning electron microscopy revealed the presence of glands, but these were specifically located on the undersurface (Williams, Harborne, & Bonner, 1998).

A parallel investigation of leaf and disc acetone washings of tansy (which lacks ray florets) revealed the

presence of some ten flavonoids Table 2. In this plant, *O*-methylated flavones dominate over *O*-methylated flavonols. In addition to scutellarein (6-hydroxyapigenin) 6-methyl ether (6), three methyl ethers of 6-hydroxyluteolin are present on the leaf surface. They are the 6-methyl ether (7), the 6,3'-dimethyl ether (8) and the 6,7,4'-trimethyl ether (9). The identities of these compounds was confirmed largely by comparison with authentic markers. In feverfew, the leaf and disc flavonoid constituents are broadly similar. However, in tansy, they are quite different, with no overlap between the two kinds of plant tissue.

2.3. Water-soluble flavonoids of feverfew and tansy

Following the removal of surface flavonoids by acetone washing, the residual tissues of feverfew and tansy were separately extracted in 80% methanol–water. The flavonoids were then separated and identified by standard procedures (Williams et al., 1995). The 7-glucuronides and 7-glucosides of apigenin and luteolin are major constituents, especially in the leaves (Table 3). There are some differences between tissues in both species. The ray florets of feverfew, for example, contain two 7-diglycosides of apigenin, based on glucuronic acid, which are not present in disc or leaf. Again, the leaf of feverfew contains chrysoeriol 7-glucuronide, not detectable in ray or

Table 3

Water-soluble flavonoids in feverfew and tansy. Key: Ap, apigenin; Lu, luteolin; Ch, chrysoeriol; Qu, quercetin; 60HLu, 6-hydroxyluteolin

	Feverfew	Tansy
Leaf ^a	Ap 7-glucuronide Lu 7-glucuronide Lu 7-glucoside Ch 7-glucuronide	Ap 7-glucoside Ap 7-glucuronide Lu 7-glucoside Lu 7-glucuronide
Disc	Ap 7-glucuronide Lu 7-glucuronide Lu 7-glucoside Qu 7-glucuronide	Lu 7-glucoside Lu 7-glucuronide 60HLu 7-glucoside Diosmetin 7-glucuronide
Ray	Ap 7-glucuronide Ap 7-diglucuronide Ap 7-glucosylglucuronide	—

^a Leaf constituents of feverfew described in Williams et al. (1995).

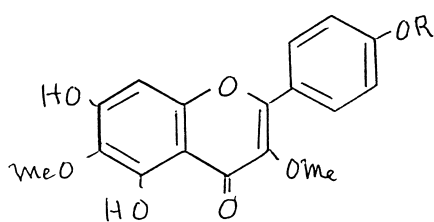
disc. The most distinctive glycoside of tansy is 6-hydroxyluteolin 7-glucoside, present in the disc flowers. It may be noted, however, that 6-hydroxyluteolin is

present in methylated form in tansy, in the leaf glands (Table 2). Tansy is also distinguished from feverfew in the presence of diosmetin 7-glucuronide in the disc flowers (Table 3).

2.4. Pharmacology of feverfew and tansy surface flavonoids

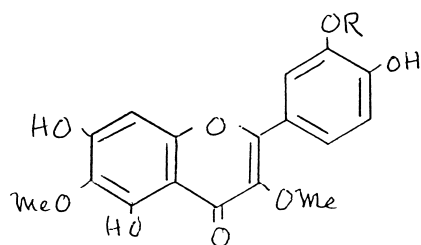
Earlier, we reported that the main feverfew flavonoid, now revised as santin, inhibited both of the major pathways of arachidonate metabolism in rat peritoneal leucocytes, these being the cyclo-oxygenase and 5-lipoxygenase pathways (Hoult et al., 1995; Williams et al., 1995). We have now tested two further 6-hydroxyflavonols from feverfew and two of the 6-hydroxyflavones from tansy. The results are shown in Table 4.

As can be seen from Fig. 1, 6-hydroxykaempferol 3,6-dimethyl ether has an equivalent profile of enzyme inhibitory activity to santin in both assays, but it is much less potent. By contrast, quercetagenin 3,6,3'-trimethyl ether shows preferential activity against cyclo-oxygenase with much less activity in inhibiting 5-lipox-



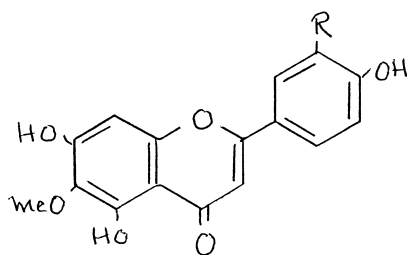
1 R = H

2 R = Me



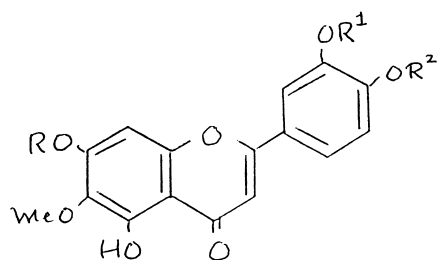
3 R = H

4 R = Me



6 R = H

7 R = OH



8 R = H, R¹ = Me, R² = H

9 R = Me, R¹ = H, R² = Me

Fig. 1. Lipophilic flavones and flavonols of *Tanacetum*.

Table 4

Relative inhibition of thromboxane B₂ and leukotriene B₄ generation by feverfew and tansy surface flavonoids. These results are based on several experiments similar to the one shown in Fig. 1, from which molar concentrations needed to inhibit product formation have been calculated

Flavonoids	IC ₅₀ values ^a for:	
	cyclo-oxygenase (thromboxane B ₂) (μM)	5-lipoxygenase (leukotriene B ₄) (μM)
<i>From feverfew leaf surface</i>		
2	27	58
1	182	182
4	22	167
<i>From tansy leaf surface</i>		
7	61	97
8	89	84

^a Concentration required to produce 50% inhibition of product formation.

xygenase. Thus, the three 6-hydroxyflavonols differ considerably in their profiles as enzyme inhibitors. Previous studies with other flavonoids and with coumarins have also shown similar variations in activity (Hoult, Moroney, & Payá, 1994; Alcaraz, & Ferrándiz, 1987; Moroney, Alcaraz, Forder, Carey, & Hoult, 1988).

The results with the tansy 6-hydroxyflavones (Table 4) show that both methyl ethers inhibit the cyclo-oxygenase and 5-lipoxygenase pathways, but appear less active as cyclo-oxygenase inhibitors than the corresponding flavonols. The results of these pharmacological experiments are also consistent with the earlier findings (Alcaraz, & Ferrándiz, 1987; Moroney, Alcaraz, Forder, Carey, & Hoult, 1988; Hoult et al., 1994) that vicinal diols yield the most active 5-lipoxygenase inhibitors: none of the present flavonoids possess this structural feature and none of them display potent or selective 5-lipoxygenase inhibition (Table 4).

Table 5

R_F and HPLC data for lipophilic 6-hydroxyflavones **6–9** from tansy

6-Hydroxyflavone methyl ether	$R_f \times 100$				HPLC R_f^a
	on cellulose in			on silica gel in toluene:HOAc (4:1)	
	30% HOAc	40% HOAc	50% HOAc		
6	25	45	57	15	6.54
7	07	21	35	06	5.33
8	21	35	53	29	6.93
9	25	45	63	37	9.49

^a C₁₈-phenyl reverse phase column at 25°C 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–H₂O, 18:1:1. UV detection at 260–350 nm.

3. Experimental

3.1. Plant material

Fresh leaves, flowers and seeds of *T. parthenium* (L.) Schultz Bip. and *T. vulgare* L. were collected from plants grown at the School of Plant Sciences, The University of Reading from seed supplied by the Botanic Gardens of Munich and Berlin-Dahlem, Germany, respectively. Voucher specimens have been deposited in the University of Reading Herbarium (RNG).

3.2. Isolation of lipophilic flavonoids from feverfew seed for NMR analysis

The flavonoids were removed from the surface of the seeds by covering with Me₂CO and immediately decanting off the solvent (×3). The concentrated extract was successively chromatographed on (1) polyamide with a H₂O/MeOH gradient ranging from 30 to 80% MeOH, (2) Sephadex LH20 with Me₂CO/MeOH/H₂O (2:1:1) and (3) Sephadex LH20 with 80% MeOH. In all systems **2** moved faster than **1** and **3** but only the third system allowed good separation of **1** and **3**. However, the other systems were essential to remove any foreign matter. The washings of ca. 200 g of seeds yielded 15 mg of **1**, 120 mg of **2** and **4** and 30 mg of **3**. Compounds **2** and **4** were separated subsequently by PPC in 30% HOAc. Details of the ¹H and ¹³C NMR analyses are given in Table 1.

3.3. Isolation of lipophilic flavonoids from leaf and flower tissue of feverfew and tansy

The flavonoids were extracted from fresh leaf and flower material by dipping the respective organs briefly in Me₂CO × 3. The ray and disc florets of feverfew were treated separately. Tansy has only disc florets. The flavonoid constituents were separated by multiple silica gel TLC in toluene:HOAc, 4:1 and PPC in 30%

HOAc followed by purification on Sephadex LH20 CC by first washing with H₂O and then elution with MeOH.

3.4. Identification of further lipophilic flavonoids from feverfew

Free apigenin, luteolin and chrysoeriol were identified by standard procedures (UV spectral data, HPLC R_f 's and co-chromatography in 50% HOAc, Forestal and CHCl₃:HOAc, 2:1 compared with authentic markers). Apigenin was confirmed by EIMS: molecular ion at 270 (required 270) with A ring fragment at 153 and B-ring fragment at 151 *m/z*. The identity of quercetageitin 3,6,3'-trimethyl ether (**4**) (for NMR data, see Table 1) from feverfew seed was confirmed by UV spectral analysis: $\lambda_{\max}$ MeOH 256, 271^{sh}, 351; + NaOAc 274, 380; + H₃BO₃ 258, 271, 353; + AlCl₃ 265, 368; + AlCl₃HCl 262, 364 and + NaOH 272, 403, which indicated free 7 and 4'-hydroxyls from the positive NaOAc and normal NaOH shifts. EIMS, R_f and HPLC data for **4** together with full data (EIMS, R_f 's, HPLC R_f 's and UV spectral data) for **1–3** have been published previously (Williams et al., 1995). Apigenin and luteolin 7-glucuronides were identified by co-chromatography with authentic markers and by comparison with samples obtained from the water soluble extracts of feverfew leaf, disc and ray florets (see Section 3.6).

3.5. The identification of lipophilic flavonoids from leaf and flower of tansy

The known aglycones: scutellarein 6-methyl ether (**6**), 6-hydroxyluteolin 6-methyl ether (**7**), 6,3'-dimethyl ether (**8**) and 6,7,4'-trimethyl ether (**9**) were identified by standard procedures compared with authentic markers. Details of R_f values and HPLC R_f 's are given in Table 5 and UV spectral data in Table 6. The identities of **6–9** were confirmed by EIMS: **6**, m/z 300 (required 300) with a fragment at 285 (M-15); **7**, m/z at 316 (required 316) with a fragment at 301 (M-15); **8**, m/z at 330 (required 330) with fragments at 315 (M-15), 312 (M-18) and 287 (M-43) and **9**, m/z 344 (required 344) with fragments at 329 (M-15), 326 (M-18) and 301 (M-43).

3.6. The identification of the water soluble flavonoids in feverfew and tansy

Known cpds: apigenin, luteolin and chrysoeriol 7-glucosides and 7-glucuronides were identified by standard procedures (UV spectral data, TLC cellulose R_f 's in BAW, BEW, H₂O and 15% HOAc and acid hydrolysis to aglycone and sugar). An apigenin 7-diglucuronide and a 7-glucosylglucuronide were partially characterised by the same procedure. Their increased mobility in aqueous solvents and reduced mobility in BAW compared with the monoglycosides and by comparison with lit. values for similar diglycosides indicated their diglycosidic nature.

3.6.1. 6-Hydroxyluteolin 7-glucoside

6-Hydroxyluteolin 7-glucoside was isolated from tansy leaf after PC in 3 solvents R_f , BAW 0.30, 15% HOAc 0.07 and had identical UV spectra and shifts to authentic material (Harborne, 1967). It co-chromatographed in 4 solvents with the marker compound, earlier isolated from leaf of *Catalpa bignonioides* (Harborne, 1967). On acid hydrolysis, it gave 6-hydroxyluteolin and glucose.

3.6.2. Apigenin 7-(glucosylglucuronide)

Apigenin 7-(glucosylglucuronide) was isolated from ray flowers of *T. parthenium*, together with apigenin 7-glucuronide. It has the typical UV spectrum and shifts as an apigenin 7-glycoside. It was slowly hydrolysed by 2 N HCl to yield apigenin, and glucose and glucuronic acid in approximately 1:1 ratio. R_f data: 0.43 in BAW, 0.34 in 15% HOAc, cf. apigenin 7-glucuronide 0.63 and 0.25, respectively. Apigenin 7-diglucuronide was also obtained from the ray flowers. It also gave a UV spectrum and shifts of an apigenin 7-glycoside. On acid hydrolysis, it was slowly hydrolysed to apigenin and glucuronic acid. R_f data: 0.40 in BAW, 0.41 in 15% HOAc, cf. apigenin 7-glucuronide 0.63 and 0.25, respectively.

3.7. Preparation of rat leukocytes and their incubation for eicosanoid generation

Mixed peritoneal leukocytes were elicited from male Wistar rats by an i.p. injection of 10 ml 6% (w/v)

Table 6

UV spectral data for lipophilic 6-hydroxyflavones **6–9** from tansy. nd, not determined; dep, depressed peak

6-Hydroxy- flavone methyl ether	$\lambda_{\max}$ MeOH	+ NaOAc	+ H ₃ BO ₃	+ AlCl ₃	+ AlCl ₃ /HCl	+ NaOH
6	273, 332	275, 378	275, 341	nd	nd	275, 396
7	257, 271, 344	257, 271, 382	258, 371	nd	nd	275, 405
8	274, 343	276, 387	276, 347	282, 362	282, 357	275, 400 dep
9	276, 341	276, 367	277, 341	283, 360	281, 356	276, 365 dep

oyster glycogen and prepared using a procedure described by Moroney et al., 1988 (Hoult et al., 1994). The cells were resuspended at a density of 2.5×10^6 cells/ml in Hanks balanced salt solution containing 1.26 mM Ca^{2+} and 0.9 mM Mg^{2+} . Cell smears showed that 70–80% of the cells were PMN neutrophils, the remainder being monocytes. Cell viability based on trypan blue exclusion was greater than 95%. Triplicate aliquots of 0.5 ml leukocytes were preincubated at 37°C for 10 min with 2.0 µl ethanol containing the compound of interest or equivalent volume of vehicle alone. To this was then added 1 µl of calcium ionophore A23187 (to give final concentration 1 µM), dissolved in DMSO (1 µl of this vehicle was added to 'basal' unstimulated control tubes lacking ionophore) for a further 10 min incubation. After the cells had been pelleted by centrifugation at 2500g for 10 min at 4°C, the supernatants were decanted and frozen. Aliquots of 2–25 µl of the thawed samples were subjected to radioimmunoassay for TXB_2 or LTB_4 as described previously (Alcaraz, & Ferrándiz, 1987; Moroney et al., 1988).

Acknowledgements

We are grateful to Mrs. Jenny Greenham for experimental assistance and to John Eagles, Food

Research Institute, Norwich, for EI-MS determinations.

References

- Alcaraz, M. J., & Ferrándiz, M. T. (1987). *J. Ethnopharmacol.*, **21**, 209–216.
- Barbera, O. (1986). *Phytochemistry*, **25**, 2357.
- Blakeman, J. P., & Atkinson, P. (1979). *Physiol. Plant Pathol.*, **15**, 183.
- Harborne, J. B. (1967). *Phytochemistry*, **6**, 1643.
- Hoult, J. R. S., Moroney, M. A., & Payá, M. (1994). *Methods Enzymol.*, **234**, 442–454.
- Hoult, J. R. S., Pang, L. H., Bland-Ward, B. A., Forder, R. A., Williams, C. A., & Harborne, J. B. (1995). *Pharm. Sci.*, **1**, 71.
- Khvorost, P. P. (1969). *Chem. Abstr.*, **71**, 19517 m.
- Markham, K. R., & Chari, V. M. (1982). In J. B. Harborne, & T. J. Mabry, (eds) *The flavonoids, advances in research* (pp. 19–134). London: Chapman and Hall.
- Markham, K. R., & Geiger, H. (1994). In J. B. Harborne, (ed.) *The flavonoids, advances in research since 1986* (pp. 441–498). London: Chapman and Hall.
- Moroney, M. A., Alcaraz, M. J., Forder, R. A., Carey, F., & Hoult, J. R. S. (1988). *J. Pharm. Pharmacol.*, **40**, 787.
- Newell, C. A., Anderson, L. A., & Phillipson, J. D. (1996). *Herbal medicines*. London: The Pharmaceutical Press.
- Ognyanov, I., & Tochorova, M. (1983). *Planta Med.*, **48**, 181.
- Williams, C. A., Hoult, J. R. S., Harborne, J. B., Greenham, J., & Eagles, J. (1995). *Phytochemistry*, **38**, 267.
- Williams, C.A., Harborne, J.B., & Bonner, L.J. (1998). Unpublished results.
- Wollenweber, E., Stüber, A., & Kraut, L. (1997). *Phytochemistry*, **44**, 1399.