Exudate Flavonoids in Some Gnaphalieae and Inuleae (Asteraceae)

Eckhard Wollenweber[a,*], Matthias Christ[a], R. Hugh Dunstan[b], James N. Roitman[c], and Jan F. Stevens[d]

[a] Institut für Botanik der TU Darmstadt, Schnittspahnstrasse 4, D-64287 Darmstadt, Germany. Fax: 0049-6151/164630. E-mail: wollenweber@bio.tu-darmstadt.de
[b] School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia
[c] Western Regional Research Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710, U.S.A.
[d] Department of Pharmaceutical Sciences, 203 Pharmacy Building, Oregon State University, Corvallis, OR 97331, U.S.A.

* Author for correspondance and reprint requests

Z. Naturforsch. **60 c**, 671–678 (2005); received May 19, 2005

Three members of the tribe Gnaphalieae and six members of the tribe Inuleae (Asteraceae) were analyzed for their exudate flavonoids. Whereas some species exhibit rather trivial flavonoids, others produce rare compounds. Spectral data of rare flavonoids are reported and their structural identification is discussed. 6-Oxygenation of flavonols is a common feature of two *Inula* species and *Pulicaria sicula.* By contrast, flavonoids with 8-oxygenation, but lacking 6-oxygenation, are common in two out of three Gnaphalieae species examined. In addition, B-ring deoxyflavonoids are abundantly present in the leaf exudates of *Helichrysum italicum* (Gnaphalieae). These distinctive features of the two Asteraceae tribes are in agreement with previous flavonoid surveys of these and related taxa.

Key words: Gnaphalieae, Inuleae, Flavonoids

Introduction

Plants belonging to the sunflower family are well-known to produce a wealth of flavonoid aglycones (Bohm and Stuessy, 2001). These are normally dissolved in a terpenoid resin that is excreted by glandular structures on their aerial parts. In the course of ongoing studies on the occurrence of exudate flavonoids in Asteraceae (see *e.g.* Wollenweber *et al.*, 1997a, b; Valant-Vetschera *et al.*, 2003), we have now analyzed three members of the tribe Gnaphalieae and six members of the tribe Inuleae (Bremer, 1994). The exudate flavonoid patterns of these plants are presented as follows.

Materials and Methods

Plant material

Asteriscus aquaticus (L.) Less. [syn.: *Nauplius aquaticus* (L.) Cass.], *Helichrysum italicum* (Roth) Don., *Helichrysum stoechas* (L.) Moench, *Helipterum strictum* (Lindl.) Benth., *Inula conyzae* (Griess.) Meikle, *Inula spiraeifolia* L., *Pulicaria sicula* (L.) Moris, and *Telekia speciosa* (Schreb.) Baumg. were cultivated in the Botanic Garden of the Technical University Darmstadt and collected in the flowering stage between October 1997 and August 2004. *Inula britannica* L. was collected in August 2000 on the bank of the river Elbe near Arneburg (Sachsen-Anhalt) by H. Groh. Vouchers were deposited in the herbarium of the Botanic Garden of the TU Darmstadt.

Extraction and isolation

Aerial parts of freshly collected flowering plants were briefly rinsed with acetone to dissolve the exudate material. The solutions were evaporated to dryness, re-dissolved in a small volume of hot MeOH, cooled to – 10 °C, and any precipitated material was separated by centrifugation. The solutions were then passed over Sephadex LH-20 (Pharmacia), and eluted with MeOH, to separate the flavonoids from the predominant terpenoids. At this point, many flavonoids were readily and unambiguously identified by direct comparison with markers. In several cases, however, further workup of flavonoid fractions was required, as well as further purification to isolate individual compounds. Relevant fractions were chromatographed over silica gel, polyamide SC-6 or acetylated polyamide (Macherey-Nagel), eluted with

0939–5075/2005/0900–0671 $ 06.00

toluene and increasing quantities of methylethyl ketone and methanol. Fractions were monitored and comparisons with markers were achieved by TLC on polyamide (DC 11, Macherey-Nagel) with the solvents i) $PE_{100-140}$/toluene/MeCOEt/MeOH 12:6:1:1 v/v/v/v, ii) toluene/PE100–140/MeCOEt/MeOH 12:6:2:1 v/v/v/v, iii) toluene/dioxane/MeOH 8:1:1 v/v/v, and iv) toluene/MeCOEt/MeOH 12:5:3 v/v/v, and on silica gel with solvents v) toluene/MeCOEt 9:1 v/v and vi) toluene/dioxane/HOAc 18:5:1 v/v/v. Chromatograms were viewed under UV light (366 nm) before and after spraying with "Naturstoffreagenz A" (1% of diphenyl-boric acid 2-aminoethyl ester in MeOH). In some cases, crude flavonoid materials were further purified to homogeneity by semi-preparative HPLC as described in Stevens *et al.* (1999). Authentic samples of flavonoids were available in E.W.'s laboratory. Isolated flavonoids were characterized by UV-VIS, MS and NMR spectra. GC-MS was applied to analyze mixtures of exudate flavonoids from *Pulicaria sicula.*

NMR and MS

Some ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker DRX 600 spectrometer at 600 MHz and 150 MHz, respectively. $^1H-^{13}C$ heteronuclear multiple bond connectivity (HMBC) spectra were recorded on this instrument using standard pulse sequences. ^1H and ^{13}C NMR spectra of a flavonoid from *Inula conyzae* were recorded in DMSO-d_6 on a Bruker AMX 400 spectrometer at 100 MHz and 400 MHz, respectively. Electron impact mass spectra were obtained on a Varian MAT 212 Spectrometer at 70 eV. Atmospheric Pressure Chemical Ionization (APCI) MS/MS spectra were recorded on a PE Sciex API III Plus triple quadrupole instrument as described in Stevens *et al.* (1999).

In the following we report spectral data for those flavonoids that could not be readily identified by co-TLC with authentic markers.

5,7-Dihydroxy-3,8-dimethoxyflavone (**1**): APCI-MS/MS (30 eV collision energy): *m/z* = 315 $[MH]^+$, 300 $[MH\text{-}CH_3]^+$, 285 $[MH\text{-}2CH_3]^+$, 257 $[285\text{-}CO]^+$, 105 $[O\equiv C\text{-}Ph]^+$. – ^1H NMR: Table I. – The substitution pattern of the A-ring was established by $^1H-^{13}C$ HMBC correlation as follows: The 5-OH resonance at δ_H 12.3 showed cross peaks with carbon signals at δ_C 155.0, 104.3 and 99.0, which were readily assigned to C-5, C-10 and C-6, respectively. H-6 interacted with C-6 (^1J coupling), C-10, C-8, C-9, C-5 and the remaining A-ring carbon atom, C-7 (δ_C 156.0). The two methoxy groups showed interactions with C-8 and δ_C 138.8 (not C-7). The latter resonance could only be assigned to C-3, and confirms a C-3 methoxyl group. Thus, the two methoxy groups were placed at C-8 and C-3 and the two free hydroxyl groups assigned to C-5 and C-7. The compound was found to be 8-hydroxygalangin-3,8-di-*O*-methyl ether (gnaphalin), identical with an authentic sample from *Nothofagus* (Wollenweber *et al.*, 2003).

6-Hydroxykaempferol-3,7,4′-tri-O-methyl ether (**2**): UV: λ_{max} (MeOH) = 336, 282; (+ $AlCl_3$) = 362, 298; (+ $AlCl_3$/HCl) = 366, 298; (+ NaOH) = 296 nm. – MS: *m/z* = 344 (100, M^+), 329 (3, $M^+\text{-}CH_3$), 325 (17), 314 ($M^+\text{-}2CH_3$), 301 (23), 295 (24), 283 (6), 258 (10), 158, (8), 135 (14). – ^1H NMR: Table I. – ^{13}C NMR: Table II. – The identity of this product was confirmed by direct comparison with a synthetic sample of 5,6-dihydroxy-3,7,4′-trimethoxyflavone (Horie *et al.*, 1989).

Table I. ^1H NMR spectroscopic data for compounds **1**–**4**, recorded in DMSO-d_6 at 400 MHz (compound **2**) or 600 MHz (compounds **1**, **3**, **4**).

H	Compound **1**	Compound **2**	Compound **3**	Compound **4**
5-OH	12.3, s	12.27, s	12.1, s	12.3, s
3-OH			9.9, s	
3-OH and 4′-OH				10.2, s and 9.6, s
6	6.31, s			
8		6.89, s		
2′ and 6′	8.03,dd, *J* = 7.9, 1.6 Hz	8.06, d, *J* = 9.2 Hz	8.18, d, *J* = 7.7 Hz	8.08, d, *J* = 8.9 Hz
3′ and 5′		7.14, d, *J* = 9.2 Hz		6.97, d, *J* = 8.9 Hz
3′, 4′ and 5′	7.63–7.58, m		7.60–7.51, m	
3 × OMe		3.91, s, 3.87, s, 3.81, s	4.03, s, 3.91, s, 3.83, s	4.02, s, 3.90, s, 3.83, s
2 × OMe	3.81, s			

Table II. ^{13}C NMR spectroscopic data for flavonols 5,7-dihydroxy-3,8-dimethoxyflavone (**1**) and 6-hydroxy-kaempferol-3,7,4′-tri-*O*-methyl ether (**2**), recorded in DMSO-d_6 at 100 MHz (compound **2**) or 150 MHz (compound **1**).

C	**1**	**2**
2	157.3	155.2
3	138.8	137.8
4	178.3	178.1
5	155.0	145.7
6	99.0	129.6
7	156.0	154.5
8	127.6	90.9
9	148.8	148.8
10	104.3	105.6
1′	131.1	122.3
2′	128.0	129.9
3′	128.9	114.2
4′	130.2	161.3
5′	128.9	114.2
6′	128.0	129.9
3-OMe	61.0	59.7
7-OMe		56.3
8-OMe	60.0	
4′-OMe		55.4

3,5-Dihydroxy-6,7,8-trimethoxyflavone (**3**): APCI-MS/MS (30 eV collision energy): *m/z* = 345 $[MH]^+$, 330 $[MH\text{-}CH_3]^+$, 315 $[MH\text{-}2CH_3]^+$, 297 $[315\text{-}H_2O]^+$, 287 $[315\text{-}CO]^+$, 272 $[287\text{-}CH_3]^+$, 105 $[O{\equiv}C\text{-}Ph]^+$. – ^1H NMR: Table I. – The identity of this flavonol was confirmed by co-TLC comparison with an authentic standard (Wollenweber *et al.*, 1993).

3,5,4′-Trihydroxy-6,7,8-trimethoxyflavone (**4**): APCI-MS/MS (30 eV collision energy): *m/z* = 361 $[MH]^+$, 346 $[MH\text{-}CH_3]^+$, 331 $[MH\text{-}2CH_3]^+$, 313 $[331\text{-}H_2O]^+$, 303 $[331\text{-}CO]^+$, 288 $[303\text{-}CH_3]^+$, 121 $[O{\equiv}C\text{-}PhOH]^+$. – The identity of this flavonol was confirmed by ^1H NMR (Table I) and co-TLC comparison with an authentic standard (Wollenweber and Roitman, 1991).

5,7-Dihydroxy-8,4′-dimethoxyflavone: APCI-MS/MS (30 eV collision energy): *m/z* = 315 $[MH]^+$, 300 $[MH\text{-}CH_3]^+$, 168 [A-ring RDA fragment minus $CH_3]^+$, 135 $[O{\equiv}C\text{-}PhOMe]^+$. – The identity of this flavone was confirmed by co-TLC with an authentic marker of isoscutellarein-8,4′-dimethyl ether (Nakayama *et al.*, 1983).

GC-MS

When the crude exudate of *Pulicaria sicula* was dissolved in boiling methanol for the de-fatting procedure, a light yellow crystalline material remained suspended. This deposit, which was soluble only in boiling acetic acid, was subjected to GC-MS analysis.

An aliquot of the flavone extract sample was transferred to a glass derivatisation tube, dried, and derivatized by first adding 100 mL 2% (w/v) of methoxyamine hydrochloride in pyridine (MOX) followed by heating at 60 °C for 30 min. Bis(trimethylsilyl)trifluoro-acetamide (BSTFA, 150 μL) was then added and the sample was heated at 100 °C for additional 60 min to form the trimethylsilyl ether derivative (TMS) (Dunstan *et al.*, 1990). Samples were then transferred into injection vials fitted with 100 μL inserts for analysis by GC-MS.

Derivatized sample components were separated using a Hewlett Packard 6890 series gas chromatograph and detected using a Hewlett Packard series 5973 mass selective detector (MSD). The data were collected and analyzed using Agilent Chemstation software with NIST™ and WILEY™ databases. The GC was fitted with a split/splitless injector and an Agilent HP-5MS capillary column (30 m × 250 μm × 0.25 μm) with a He flow of 0.5 mL min^{-1}. The GC-MS unit was operated with the following settings: injector temperature 280 °C, temperature program 80–300 °C at 3 °C min^{-1}, with a 2 min hold at 80 °C and a 10 min hold at 300 °C. The mass spectrometer scanned from 40–650 atomic mass units at 2.44 scans per second.

Peaks at the retention times (t_R) 74.22 min, 75.26 min, 75.7 min, 76.07 min, 77.07 min, 77.51 min, 77.95 min and 78.40 min exhibited mass spectra indicating flavonoids.

t_R = 78.40 min: The molecular mass *m/z* = 518 indicates the silyl derivative of a dihydroxy-tetramethoxy-flavone. Comparative TLC with markers showed, in fact, the presence of quercetagetin-3,6,7,3′-tetramethylether (queg-3,6,7,3′-tetraMe3) and queg-3,7,3′,4′-tetraMe. The retention time t_R = 77.07 min applies to the trimethylsilyl derivative of the latter product. t_R = 78.40 min should, therefore, correspond to queg-3,6,7,3′-tetraMe.

t_R = 77.95 min: *m/z* = 576 indicates the silyl derivative of a trihydroxy-trimethoxy-flavone. A total of five compounds was identified that meets this requirement in various flavonoid fractions: quercetagetin-3,6,7-triMe, queg-3,7,3′-triMe, queg-3,7,4′-triMe, queg-3,3′,4′-triMe and queg-7,3′,4′-triMe. Queg-3,7,4′-triMe and queg-7,3′,4′-triMe were excluded since their triTMS derivatives appear at different retention times (77.6 and 76.6 min, respectrively), and queg-3,7,3′-triMe is

the only one that was identified in the relevant sample by TLC. The retention time t_R = 77.95 min was hence ascribed to the trimethylsilyl derivative of queg-3,7,3′-triMe.

t_R = 77.51 min: Unidentified.

t_R = 77.07 min: Based on comparative GC-MS, this peak corresponds to quercetagetin-3,7,3′,4′-tetramethylether (*m/z* = 518, base peak at *m/z* = 503).

t_R = 76.07 min: The mass spectra shows a molecular mass of *m/z* = 402 and the base peak at *m/z* = 387, suggesting. a hexamethoxyflavone. A search in the Wiley^{TM} database revealed it to be queg-3,5,6,7,3′,4′-hexaMe. This identification was confirmed by direct comparison with a synthetic sample (Horie *et al.*, 1989). We later managed to isolate this product and found its ^{1}H NMR data to match those reported in the literature (Ahmed *et al.*, 1989).

t_R = 75.7 min: Quercetagetin-3,6,7,3′,4′-pentamethylether was identified by comparative GC-MS with an authentic sample.

t_R = 75.26 min and t_R = 74.22 min: In both cases, an important [M-OTMS]$^+$ peak indicated a 2′-hydroxy flavonoid. Relevant compounds were not found in the Wiley^{RM} database nor in E.W.'s collection of markers. The product appearing at t_R = 74.22 min exhibits a molecular ion at *m/z* = 490 for the mono-TMS derivative of a monohydroxyhexamethoxy flavone. It was assumed to be either 2′-hydroxyquercetagetin-3,5,6,7,3′,4′-hexamethylether or 2′-hydroxy-3,5,6,7,4′,5′-hexamethoxyflavone. These compounds can be distinguished by their NMR spectra.

We did in fact isolate a small amount of a crystalline mixture of quercetagetin hexamethylether with about 20% of the product in question, and the signals of the unknown product were discernable: ^{1}H NMR: δ (ppm) = 3.86, s, 3H (OMe), 3.91, s, 3H (OMe), 3.92, s, 6H (2 × OMe), 3.95, s, 3H (OMe), 3.99 (s, 3H, OMe), 6.61 (s, 1H, H-3′), 6.73 (s, 1H, H-8), 7.1 (s, 1H, H-6′), 7.99 (s, 1H, O-H). Thorough evaluation of the NMR data and comparison with literature data (Ahmed *et al.*, 1989; Iinuma *et al.*, 1986) allows to deduce the structure of 2′-hydroxy-3,5,6,7,4′,5′-hexamethoxy flavone. The proton spectrum of the isomeric 2′-hydroxy quercetagetin-3,5,6,7,3′,4′-hexamethylether would exhibit two doublet signals, due to coupling of the 5′ and 6′-protons, whereas our product exhibits two singlets. 8-OMe-substitution, on the other hand, is excluded by comparison of the signals of the A-ring protons. In the NMR spectrum of our sample, we observed only 1 signal for the A-ring protons. Since one component of the sample is queg-3,5,6,7,3′,4′-hexaMe, H-6 of a 8-OMe compound would resonate ats lightly higher frequency. 8-OMe-substitution is thus excluded. Admittedly, confirmation of our finding by analysis of a pure sample is desirable.

The GC peak at t_R = 75.26 min corresponds to the trimethylsilyl derivative of a dihydroxy-pentamethoxy flavone, bearing one of the OH-groups at C-2′, but its identity could not be confirmed.

Results and Discussion

From nine species of Asteraceae, belonging to the tribes Gnaphalieae and Inuleae, we analyzed the externally accumulated flavonoids. Their profiles are presented in Table III. These results are briefly discussed in the following.

Inuleae

Asteriscus

A. aquaticus does not exhibit a typical resinous exudate. Only trace amounts of a few trivial flavonoids were found to be accumulated externally. Ahmed *et al.* (1991) reported quercetagetin-3′,4′-diMe and queg-3,6,3′-triMe (jaceidin) as constituents of *Asteriscus graveolens*, in addition to tissue-derived flavonoid glycosides. We assume that these two aglycones are exudate constituents, since our own studies also revealed the presence of three quercetagetin methyl ethers in the exudate of *A. sericeus* (Wollenweber *et al.*, 1997a).

Inula

The occurrence of 6-hydroxykaempferol-3,7,4′-trimethylether in nature has been reported only once previously. It was first reported from *Pulicaria dysenterica* (Williams *et al.*, 2000; no spectral data given). An earlier report from *Tanacetum parthenium* was revised to 6-OH-kae-3,6,4′-triMe (Williams *et al.*, 1999), and hence the trivial name tanetin is obsolete.

6-*O*-methylated flavones and flavonols are widespread as aglycones in the genus *Inula* (cf. Bohm and Stuessy, 2001, pp. 320–321, and references therein; Valant-Vetschera and Wollenweber, 2005). The present findings thus fit well with the known exudate flavonoid patterns of *Inula* species, where 6-*O*-derivatives dominate, and 8-*O*-deriva-

Table III. Exudate flavonoids of Inuleae and Gnaphalieae (OH, hydroxy; Me, methyl ether; OMe, methoxy; *, identified by MS and/or NMR data).

	Inuleae						Gnaphalieae		
	Asteriscus aquaticus	*Inula britannica*	*Inula conyzae*	*Inula spiraeifolia*	*Pulicaria sicula*	*Telekia speciosa*	*Helichrysum italicum*	*Helichrysum stoechas*	*Helipterum strictum*
Apigenin	×								×
Ap-7-Me (genkwanin)	×								
Ap-4′-Me (acacetin)									×
Scutellarein-6-Me (hispidulin)									
Isoscutellarein-8-Me*									×
Isoscut-8,4′-diMe* (bucegin)									×
Isoscut-7,8,4′-triMe									×
Luteolin	×	×							×
6-Hydroxyluteolin-6-Me (nepetin)		×							×
Galangin							×		
Gal-3-Me							×		
8-Hydroxygalangin							×		
8-OH-gal-3-Me*							×		
8-OH-gal-3,8-diMe* (gnaphaliin)							×		
Kaempferol	×								
Kae-3-Me (isokaempferid)							×		
Kae-4′-Me (kaempferid)			×						
3,5-DiOH-6,7,8-triOMe flavone*								×	
6-Hydroxykaempferol-6-Me						×			
6-OH-kae-3,6-diMe						×			
6-OH-kae-6,4′-diMe (betuletol)			×						
6-OH-kae-3,6,7-triMe (penduletin)				×					
6-OH-kae-3,7,4′-triMe*			×						
6-OH-kae-6,7,4′-triMe (mikanin)			×						
6-OH-kae-3,6,7,4′-tetraMe			×		×				
Herbacetin-3-Me							×		
Quercetin		×			×				
Qu-3-Me		×					×		
Qu-3,3′,4′-triMe					×				
3,5,4′-TriOH-6,7,8-triOMe flav.*								×	
Quercetagetin-6-diMe (patuletin)						×			
Queg-3,6-diMe (axillarin)		×		×					
Queg-3,7-diMe (tomentin)		×		×					
Queg-3,6,7-triMe (chrysosplenol D)				×	×				
Queg-3,7,3′-triMe (chrysosplenol C)					×				
Queg-3,7,4′-triMe (oxyayanin B)					×				
Queg-7,3′,4′-triMe					×				
Queg-3,6,7,3′-tetraMe (chrysosplenetin)				×	×				
Queg-3,6,7,4′-tetraMe (casticin)				×					
Queg-3,7,3′,4′-tetraMe					×				
Queg-3,6,7,3′,4′-pentaMe (artemetin)				×	×				
Queg-3,5,6,7,3′,4′-hexaMe					×				
Gossypetin-3-Me*							×		
Goss-3,8-diMe							×		
2′-Hydroxy-3,5,6,7,4′,5′-hexa-OMe*					×				
Pinocembrin							×		

tives are absent, as is typical in general for the tribe Inuleae s.str. (cf. Bohm and Stuessy, 2001, p. 430, Table 20.11). The *Inula britannica* plants herein analyzed, obtained from the banks of the Elbe river, exhibit a flavonoid pattern that is markedly different from that we reported previously, from plant material collected in Iran (Wollenweber *et al.*, 1997a). *Inula viscosa*, which produces flavanones and dihydroflavonols, is exceptional (Wollenweber *et al.*, 1991), a fact that might underline its affiliation as *Dittrichia viscosa*.

Pulicaria

In *Pulicaria sicula*, we found a surprising array of quercetagetin derivatives. Queg-3,5,6,7,3′,4′-hexaMe is a well-known constituent of the peel oil of *Citrus* fruits (first report: Tatum and Berry, 1972), but has otherwise been found as a natural product only in aerial parts of *Jasiona montana* (Ahmed *et al.*, 1989) and *Pallenis spinosa* (Ahmed *et al.*, 1992), along with further lipophilic flavonol aglycones (both Asteraceae, Inuleae). An earlier erroneous report on quercetagetin-7,3′,4′-triMe from *Citrus medica* revised after synthesis had shown that the reported flavone was different (Tominaga and Horie, 1993). This is therefore the first report on the natural occurrence of queg-7,3,4′-triMe (identified by direct comparison with the synthetic product). 2′-Hydroxy-3,5,6,7,4′,5′-hexamethoxyflavone has been found previously neither in nature, nor reported as a synthetic product.

Eight of approximately 80 species of *Pulicaria*, including the present species, have thus far been shown to exhibit flavonoid aglycones (cf. Bohm and Stuessy, 2001, p. 326; Valant-Vetschera and Wollenweber, 2005). Many of these are flavonols, mostly 6-*O*-methylated; only three are 6-*O*-methyl flavones. Flavanones and dihydroflavonols have been found in three species (cf. Bohm and Stuessy, 2001, p. 326).

Telekia speciosa

Minor amounts of only three flavonols were found in this species, the only in its genus. No previous report of its flavonoids is known.

Gnaphalieae

Helichrysum italicum

The major flavonoid of *H. italicum* is 8-hydroxygalangin-3-Me accompanied by lesser amounts of galangin, galangin-3-Me, 8-hydroxygalangin-3,8-diMe and only a trace of 8-hydroxygalangin. Further flavonols are kaempferol-3-Me, herbacetin-3-Me, quercetin-3-Me, gossypetin-3-Me, goss-3,8-diMe, and pinocembrin. 8-Hydroxygalangin is a very rare compound, found previously, *e.g.* in the exudate of *Ozothamnus ledifolius*, also as a trace constituent (Wollenweber *et al.*, 1997c). Its 3-methyl ether has been more rarely encountered, whereas the 3,8-dimethyl ether (gnaphalin) has been found more often – *e.g.* in *Gnaphalium* and in *Helichrysum.* Herbacetin-3-Me is again a relatively rare flavonol, found in some Asteraceae. Gossypetin-3-Me has been found only once previously (Roitman and James, 1985). But gossypetin-3,8-diMe is a typical flavonol of the Gnaphalieae (cf. Valant-Vetschera and Wollenweber, 2005).

Helichrysum stoechas

The flavonoid fraction of this plant's exudate contains two major flavonols, which on thin layer chromatograms are accompanied by several polar tailing spots of unknown composition. 3,5-Dihydroxy-6,7,8-trimethoxy flavone is known from *Gnaphalium*, *Helichrysum*, and two further Asteraceae. 3,5,4′-Trihydroxy-6,7,8-trimethoxy flavone has been reported as a natural flavonol only once previously, in the farinose frond exudate of the fern *Cheilanthes argentea* (Wollenweber and Roitman, 1991).

Helichrysum, a genus with some 500 species, is a rich source of flavonoid aglycones, among which all classes of flavonoids are represented. Chalcones and dihydrochalcones are abundant and struturally diverse. Flavanones are also widespread, and like the chalcones, they comprise many *C*-prenyl derivatives. Lack of B-ring substitution and the presence of 6- and/or 6-*O*-substitution are also characteristic features of *Helichrysum* flavonoids. The flavones and flavonols reported here fall into this substitution pattern.

Helipterum strictum

Isoscutellarein derivatives occur scattered among the angiosperms, and the 7,8,4′-triMe is a rather rare flavone (cf. Valant-Vetschera and Wollenweber, 2005). Two other species of *Helipterum* (syn.: *Rhodanthe*) were earlier found to produce scutellarein-6-Me (hispidulin) and 6-methoxyluteolin (eupafolin), respectively (cf. Bohm and Stuessy, 2001, p. 327).

This survey of leaf surface flavonoids reveals differences in oxygenation patterns between Inuleae and Gnaphalieae (Table III). 6-Oxygenation of flavonols is a common feature of two *Inula* species and *Pulicaria sicula.* By contrast, flavonoids with 8-oxygenation, but lacking 6-oxygenation, are common in two out of three Gnaphalieae species examined. In addition, B-ring deoxyflavonoids are abundantly present in the leaf exudates of *Helichrysum italicum* (Gnaphalieae). These distinctive features of the two Asteraceae tribes are in agreement with previous flavonoid surveys of these and related taxa (Bohm and Stuessy, 2001, p. 432–433).

Acknowledgements

E. W. wishes to thank Klaus Blümler, Helmut Groh and Dr. Stefan Schneckenburger (Botanischer Garten der TU Darmstadt) for providing the plant material. Thanks are also extended to Marion Dörr, Darmstadt, and Tony Rothkirch, Callaghan, for technical assistance. Financial support by the Deutsche Forschungsgemeinschaft (to E. W.) is gratefully acknowledged. APCI-MS/MS measurements were conducted in the Mass Spectrometry Core of the Environmental Health Sciences Center at Oregon State University (NIH grant P30 ES00210).

Ahmed A. A., Ashraf A., and Mabry T. J. (1989), Flavonoid aglycones from *Jasiona montana*. Phytochemistry **28**, 665–667.

Ahmed A. A., Ishak M. S., Michael H. N., El-Ansari M. A., and El-Sissi H. I. (1991), Flavonoids of *Asteriscus graveolens*. J. Nat. Prod. **54**, 1092–1093.

Ahmed A. A., Spaller M., and Mabry T. (1992), Flavonoids of *Pallenis spinosa* (Asteraceae). Biochem. Syst. Ecol. **20**, 785–786.

Bohm B. A. and Stuessy T. F. (2001), Flavonoids of the sunflower family (Asteraceae). Springer, Wien and New York.

Bremer K. (1994), Asteraceae. Cladistics and Classification. Timber Press, Portland, Oregon.

Dunstan R. H., Smillie R. H., and Grant B. R. (1990), The effects of sub-toxic levels of phosphonate on the metabolism and potential virulence factors of *Phytophthora palmivora*. Physiol. Mol. Plant Path. **36**, 205–220.

Horie T., Kwamura Y., Tsukayama M., and Yoshizaki S. (1989), Studies of the selective *O*-alkylation of flavonoids. XII. A new, convenient method for synthesizing 3,5-dihydroxy-6,7-dimethoxyflavones from 3,5,6,7-tetramethoxyflavones. Chem. Pharm. Bull. **37**, 1216–1220.

Iinuma M., Matoba Y., Tanak T., and Mizuno M. (1986), Flavonoids synthesis. I. Synthesis and spectroscopic properties of flavones with two hydroxy and five methoxy groups at C-2′,3′,4′,5′,6,6′,7 and 2′,3,4′,5,5′,6,7. Chem. Pharm. Bull. **34**, 1656–1662.

Nakayama M., Horie T., Lin C.-N., Arisawa M., Shimizu M. M. and Morita N. (1983), The synthesis of 5,7-dihydroxy-4′,8-dimethoxyflavone: Revised structure for cirsitakaogenin and cirsitakaoside. Yakugaku Zasshi **103**, 675–678.

Roitman J. N. and James L. F. (1985), Chemistry of toxic range plants. Highly oxygenated flavonol methyl ethers from *Gutierrezia microcephala*. Phytochemistry **24**, 835–848.

Stevens J. F., Ivancic M., Deinzer M. L., and Wollenweber E. (1999), A novel 2-Hydroxyflavanone from *Collinsonia canadensis*. J. Nat. Prod. **62**, 392–394.

Tatum J. H. and Berry R. E. (1972), Six new flavonoids from *Citrus*. Phytochemistry **11**, 2283–2288.

Tominaga H. and Horie T. (1993), Studies of the selective *O*-alkylation and dealkylation of flavonoids. XV. A convenient synthesis of 3,5,6-trihydroxy-7-methoxyflavones and revised structures of two natural flavones. Bull. Chem. Soc. Jpn. **66**, 2668–2675.

Valant-Vetschera K. M. and Wollenweber E. (2005), Flavones and flavonols. In: Flavonoids: Chemistry, Biochemistry and Applications (Andersen O. M. and Markham K. R., eds.). CRC-Press, Boca Raton.

Valant-Vetschera K. M., Fischer R., and Wollenweber E. (2003), Exudate flavonoids in species of *Artemisia* (Asteraceae-Anthemideae): new results and chemosystematic interpretation. Biochem. Syst. Ecol. **31**, 487–498.

Williams C. A., Harborne J. B., Geiger H., and Hoult J. R. S. (1999), Flavonoids of *Tanacetum parthenium* and *T. vulgare* and their anti-inflammatory properties. Phytochemistry **51**, 417–423.

Williams C. A., Harborne J. B., and Greenham J. (2000), Geographical variation in the surface flavonoids of *Pulicaria dysenterica*. Biochem. Syst. Ecol. **28**, 679–687.

Wollenweber E. and Roitman J. N. (1991), New frond exudate flavonoids from cheilanthoid ferns. Z. Naturforsch. **46c**, 325–330.

Wollenweber E., Mayer K., and Roitman J. N. (1991), Exudate flavonoids of *Inula viscosa*. Phytochemistry **30**, 2445–2446.

Wollenweber E., Fritz H., Henrich B., Jakupovic J., Schilling G., and Roitman J. N. (1993), Rare flavonoid aglycons from *Anaphalis margaritacea* and two *Gnaphalium* species. Z. Naturforsch. **48c**, 420–424.

Wollenweber E., Dörr M., Fritz H., and Valant-Vetschera K. M. (1997a), Exudate flavonoids in several Asteroideae and Cichorioideae (Asteraceae). Z. Naturforsch. **52c**, 137–143.

Wollenweber E., Dörr M., Fritz H., Papendieck S., Yatskievych G., and Roitman J. N. (1997b), Exudate flavonoids in Asteraceae from Arizona, California, and Mexico. Z. Naturforsch. **52c**, 301–307.

Wollenweber E., Dörr M., Beyer M., Roitman J. N., and Puttock C. (1997c), Rare flavonoids from *Odixia* and *Ozothamnus* spp. (Astereaceae, Gnaphalieae). Z. Naturforsch. **52c**, 571–576.

Wollenweber E., Stevens J. F., Dörr M., and Rozefelds A. C. (2003), Taxonomic significance of flavonoid variation in temperate species of *Nothofagus*. Phytochemistry **62**, 1125–1131.