Chemodiversity of Exudate Flavonoids in Seven Tribes of Cichorioideae and Asteroideae (Asteraceae)[§]

Karin M.Valant-Vetschera[a] and Eckhard Wollenweber[b,*]

[a] Department of Plant Systematics and Evolution – Comparative and Ecological Phytochemistry, University of Vienna, Rennweg 14, A-1030 Wien, Austria
[b] Institut für Botanik der TU Darmstadt, Schnittspahnstrasse 3, D-64287 Darmstadt, Germany. E-mail: wollenweber@bio.tu-darmstadt.de

* Author for correspondence and reprint requests

Z. Naturforsch. **62c**, 155–163 (2007); received October 26/November 24, 2006

Members of several genera of Asteraceae, belonging to the tribes *Mutisieae*, *Cardueae*, *Lactuceae* (all subfamily Cichorioideae), and of *Astereae*, *Senecioneae*, *Helenieae* and *Heliantheae* (all subfamily Asteroideae) have been analyzed for chemodiversity of their exudate flavonoid profiles. The majority of structures found were flavones and flavonols, sometimes with 6- and/or 8-substitution, and with a varying degree of oxidation and methylation. Flavanones were observed in exudates of some genera, and, in some cases, also flavonol- and flavone glycosides were detected. This was mostly the case when exudates were poor both in yield and chemical complexity. Structurally diverse profiles are found particularly within *Astereae* and *Heliantheae*. The tribes in the subfamily Cichorioideae exhibited less complex flavonoid profiles. Current results are compared to literature data, and botanical information is included on the studied taxa.

Key words: Asteraceae, Exudates, Flavonoids

Introduction

The family of Asteraceae is distributed worldwide and comprises 17 tribes, of which *Mutisieae*, *Cardueae*, *Lactuceae*, *Vernonieae*, *Liabeae*, and *Arctoteae* are grouped within subfamily Cichorioideae, whereas *Inuleae*, *Plucheae*, *Gnaphalieae*, *Calenduleae*, *Astereae*, *Anthemidae*, *Senecioneae*, *Helenieae*, *Heliantheae* and *Eupatorieae* are members of subfamily Asteroideae. The subfamily Barnadesioideae consists of a few genera only, and it is assumed to be basal in the Asteraceae (Bremer, 1994). Alignment of genera to the existing tribes or subtribes is sometimes difficult, and in several cases, still heavily discussed. Much information on the phylogeny of the family is now coming from molecular systematic studies. Also chemical constituents are seen as valuable additional characters, such as flavonoids at the generic level (*e.g. Artemisia*: Belenovskaya, 1996) and even at the family level (Emerenciano *et al.,* 2001). However, phytochemical variation may be much larger than variation at the molecular genetic level. Therefore, comparison of accumulation trends in terms of substitution patterns is more indicative for chemodiversity than single compounds.

Earlier, we have shown that some accumulation tendencies apparently exist in single tribes (Wollenweber and Valant-Vetschera, 1996). In continuation of such studies (Wollenweber *et al.,* 1989; 1997a, b; 2005), species belonging to various tribes have been analyzed for the first time for exudate flavonoids, and their accumulation trends are discussed in relation to previously published data, both on exudate and on tissue flavonoids, and in relation to available botanical information.

Material and Methods

Collection data

Eriophyllum lanatum var. *lanatum, Euryops acraeus, Grindelia robusta, Haplopappus glutinosus, Hypochaeris maculata, Hypochaeris radicata, Hypochaeris uniflora*, *Iva xanthifolia, Sigesbeckia flocculosa, Tonestus lyallii, Xanthium strumarium, Xeranthemum foetidus,* and *Zinnia elegans* were cultivated in the Botanic Garden of the Technical University Darmstadt (BG-TUD) and collected in the flowering stage between October 1997 and Au-

[§] Part V in the series "Exudate Flavonoids in Miscellaneous Asteraceae". For Part IV see Wollenweber *et al.* (2005), for Part III see Wollenweber *et al.* (1997b).

0939–5075/2007/0300–0155 $ 06.00

gust 2004. Vouchers are deposited in the Herbarium of BG-TUD and in the Herbarium in the Institute of Botany, University of Vienna (WU) and partly in Herbaria of collectors (Missouri, MO). Plants collections from natural habitats are listed below.

Balsamorhiza sagittata: Bogus Canyon, North Logan, Cache County, Utah, USA (B. Bohm, spring 2000, UBC). Further material of *B. sagittata* was collected from four populations in British Columbia, Canada (Ecological Reserve, Princeton; Anarchist Mountain, Ossyos; Okanogan Falls; Marble Canyon area; UBC).

Balsamorhiza macrophylla: Bogus Canyon, North Logan, Cache County, Utah, USA (B. Bohm, spring 2000, UBC).

Gochnatia foliolosa: San Felipe, Precordillera (P. López, December 2001, CONC).

Gochnatia glutinosa: Argentina, 10 km W of junction routes 9 and 52, ca. 20 km N of Volcan, 2450 m (Stuessy 12978, 20/02/93, WU).

Grindelia chiloensis: Argentina, 12 km W of junction routes 3 and 26, Patagonican Steppe, S of Comodoro, 220 m (Stuessy 12913, 15/02/93, WU).

Gutierrezia resinosa:

a) Chile, Region IV, 22.6 km N Ovalle, on road to La Serena, 400 m (Stuessy 12753, 18/01/93, WU).

b) Chile, Region IV, ca. 2 km S of junction gravel roads toward Andacollo and Corral Quemada, 610 m (Stuessy 12764: 19/01/93 (WU).

Hazardia berberidis: Arizona (D. W. Clark, 1595, ASU).

Hazardia ferrisiae: Arizona (D. W. Clark, 1606, ASU).

Hazardia orcuttii: Arizona (D. W. Clark, 1612, ASU).

Hieracium intybaceum: Summit station of mount Rubiei, near Lecco at Lake Como, Italy (E. Wollenweber, September 1999, BG-TUD).

Lapsana communis: Field-collected at Münster, near Darmstadt (H. Groh, June 2001, BG-TUD).

Nardophyllum scoparium: Chile, 9.6 km N of Hurtado on winding gravel road to Uicuna, 1750 m (Stuessy 1268, 19/01/93, WU).

Olearia glutinosa: Tasmania (Rozefelds 1378, 1999, HO).

Olearia ramulosa: Tasmania (Rozefelds 1379, 1999, HO).

Proustia cuneifolia: San Felipe, Precordillera (P. López, Dec. 2001, CONC).

Senecio murinus: Chile, 6.9 km NE of junction gravel roads toward Andacollo and Corral Quemada (T. Stuessy 19/01/93, WU).

Silphium laciniatum: Seeds from Tucker Prairie Natural Area, Callaway Co., Missouri (K. M. Valant-Vetschera, August 1999, SCHN 5962, BG-TUD)

Silphium terebinthinaceum: Missouri, 1.8 km S of State Highway 72 junction on State Highway 21, just S of Arcadia town limits (G. Yatskievych and T. E. Smith, 99–152, 10. 09. 1999, MO).

Sonchus arvensis: Field-collected at Münster, near Darmstadt (H. Groh, July 1997, BG-TUD).

Extraction and identification

Aerial parts were collected either in the field and thoroughly air-dried, or they were freshly collected in the Botanic Garden of TU Darmstadt. Both kinds of material were rinsed with acetone very briefly, to avoid extraction of tissue constituents. The mostly resinous residues obtained after evaporation of acetone were "defatted" by solution in a small volume of hot MeOH, cooling to −10 °C, and removal of precipitated material by centrifugation. The supernatants were chromatographed on a Sephadex LH-20 column (Pharmacia), eluted with methanol, to separate flavonoids from the predominant terpenoids. At this point, most flavonoids were readily and unambiguously identified by direct comparisons with markers.

In some cases, however, further workup of flavonoid fractions by column chromatography over silica, polyamide SC-6 or acetylated polyamide (Macherey-Nagel; elution with toluene and increasing quantities of methylethyl ketone and methanol) was required. Several flavonoids were further purified by preparative TLC on silica. Comparative TLC of fractions and co-chromatography with markers were carried out 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/$PE_{100-140}$/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 with the 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" (0.2% of diphenyl-boric acid 2-aminoethyl ester in MeOH). Authentic samples of flavonoids were available in E. W.'s laboratory. Some flavonoids were further characterized by their mass spectra.

Results

The analyzed species are grouped according to their sectional alignment (Bremer, 1994). Results concern genera of *Mutisieae*, *Cardueae*, *Lactuceae* of subfamily Cichorioideae, and *Astereae*, *Senecioneae*, *Helenieae*, and *Heliantheae* of subfamiliy Asteroideae. Their aglycone composition is listed in sequence of increasing complexity of substitution patterns and in abbreviated form (see Table I). Hydroxylation is indicated as OH, methoxylation as OMe, and methyl groups are abbreviated as Me. Compounds listed in brackets were present only in minor amounts. The various accumulation trends are presented according to the tribal alignment of genera (Bremer, 1994). It should be mentioned that aglycones reported in the literature as originating from leaf material most probably occur as exudate constituents.

Flavonoid aglycones of Mutisieae species

From this tribe, species of the S-hemispheric *Gochnatia* and *Proustia* have been analyzed. *Gochnatia* comprises 68 South American species, with 2 species occurring in Southeast Asia. They are mostly shrubs or trees. Five species are summarized in *Proustia*, occurring in South America

Table I. Flavonoid aglycones: structural information, trivial names and abbreviations used in the text.

Flavonoid structure	Trivial name	Abbreviated name
5,7,4′-TriOH-flavone	Apigenin	ap
	Genkwanin	ap-7-Me
	Acacetin	ap-4′-Me
5,6,7,4′-TetraOH-flavone	Scutellarein	scut
	Pectolinarigenin	scut-6,4′-diMe
5,7,3′,4′-TetraOH-flavone	Luteolin	lut
	Chrysoeriol	lut-3′-Me
	Diosmetin	lut-4′-Me
	Velutin	lut-7,3′-diMe
5,6,7,3′,4′-PentaOH-flavone	6-OH-Luteolin	6-OH-lut
	Nepetin	6-OH-lut-6-Me
	Eupalitin	6-OH-lut-6,3′,4′-triMe
3,5,7,4′-TetraOH-flavone	Kaempferol	kae
	Isokaempferide	kae-3-Me
	Rhamnocitrin	kae-7-Me
	Kumatakenin	kae-3,7-diMe
	Ermanin	kae-3,4′-diMe
3,5,6,7,4′-PentaOH-flavone	6-OH-Kaempferol	6-OH-kae
	Penduletin	6-OH-kae-3,6,7-triMe
3,5,7,3′,4′-PentaOH-flavone	Quercetin	qu
	Rhamnetin	qu-7-Me
	Isorhamnetin	qu-3′-Me
	Rhamnazin	qu-7,3′-diMe
	Ombuin	qu-7,4′-diMe
	Pachypodol	qu-3,7,3′-triMe
	Ayanin	qu-3,7,4′-triMe
	Retusin	qu-3,7,3′,4′-tetraMe
3,5,6,7,3′,4′-HexaOH-flavone	Quercetagetin	queg
	Patuletin	queg-6-Me
	Axillarin	queg-3,6-diMe
	Tomentin	queg-3,7-diMe
	Spinacetin	queg-6,3′-diMe
	Chrysosplenol-D	queg-3,6,7-triMe
	Jaceidin	queg-3,6,3′-triMe
	Centaureidin	queg-3,6,4′-triMe
	Chrysosplenetin	queg-3,6,7,3′-tetraMe
	Bonanzin	queg-3,6,3′,4′-tetraMe
3,5,7,8,3′,4′-HexaOH-flavone	Gossypetin	goss
5,7,4′-TriOH-flavanone	Naringenin	nar
	Sakuranetin	nar-7-Me
	Isosakuranetin	nar-4′-Me
5,7,3′,4′-TetraOH-flavanone	Eriodictyol	eriod

and being more or less spiny shrubs. *Gochnatia* is claimed to be a taxon crucial for understanding the evolution of the *Mutisieae* (Bremer, 1994).

1.) *Gochnatia foliolosa* D. Don ex Hook and Arn.: ap/-7-Me; kae-7-Me/-3,7-diMe; qu/-3-Me/-7-Me/-3,7-diMe/-3,3′-diMe/-7,3′-diMe/-3,7,4′-triMe (; eriod).

2.) *Gochnatia glutinosa* D. Don ex Hook and Arn.: kae-3-Me/-3,7-diMe/-3,4′-diMe/-7,4′-diMe/-3,7,4′-triMe; qu/-3-Me/-7-Me/-3′-Me/-3,7-diMe/-3,3′-diMe; eriod/-7-Me.

3.) *Proustia cuneifolia* D. Don.: ap-7-Me/-4′-Me; kae-7-Me; qu-7,3′-diMe; nar-7-Me. Earlier, nar-7-Me, nar-4′-Me, ap-7,4′-diMe have been isolated from another accession (Bittner *et al.,* 1989).

Exudate flavonoids have so far not been reported for both genera. Earlier studies on *G. foliolosa* var. *fascicularis* yielded kae-3,7-diMe, qu-3′-Me, qu-3,7-diMe and qu-3,3′-diMe (Faini *et al*., 1984). Ybarra *et al.* (1994) reported on the occurrence of ap-7-Me, nar-7-Me, eriod-7Me, kae-3,3′-diMe and kae-3,7-diMe in aerial parts of *G. glutinosa*. Both species are the only members of *Gochnatia* sect. *Pentophorus*, sharing glandular lower leaf surfaces as typical morphological feature, which distinguishes them from species of other sections (Freire *et al.,* 2002). Leaf extracts of *Gochnatia polymorpha* (Less.) Cabr. var. *polymorpha*, which is placed in a different section (Freire *et al.,* 2002), were earlier found to contain ap-7-Me and 6OH-lut-6,4′-diMe (desmethoxycentaureidin; Sacilotto *et al*., 1997). Activity-guided fractionation of aerial part extracts of *Proustia pyrifolia* DC. yielded quercetin and dihydroquercetin (Delporte *et al.,* 2005).

Flavonoid aglycones of Cardueae species

The small genus *Xeranthemum* with its 5 mostly annual species is distributed from South Europe to Southwest Asia and North Africa (Bremer, 1994). Exudate flavonoids were so far unknown from this genus. Aerial parts of *X. annuum* were earlier found to accumulate luteolin and quercetin (Zemtsova and Molchanova, 1979). *Xeranthemum foetidus* analyzed now yielded only quercetin, apigenin and scut-6-Me, along with traces of kaempferol.

Flavonoid aglycones of Lactuceae species

Species of the genera *Hieracium*, *Hypochaeris*, *Lapsana* and *Sonchus* were included in our comparison of exudate flavonoids. All of them are annual or perennial herbs. *Hieracium* comprises perennial herbs with circumpolar, but European-centred distribution. *Hypochaeris*, by contrast, occurs also in the Mediterranean and in South America, and comprises some 60 species. *Lapsana* is much smaller, with 10 species being distributed in Europe and temperate Asia, as well as in Northwest Africa. Finally, *Sonchus* with its 60 species has a world-wide distribution (Bremer, 1994).

1.) *Hieracium intybaceum* All.: nar-7-Me/-7,4′-diMe; ap/-7-Me/-7,4′-diMe. Earlier, ap-4′-Me has been described as further exudate constituent from another accession (Wollenweber, 1984). This indicates that some variation exists in this alpine plant species. A similar composition of exudate flavonoids was also reported from *H. amplexicaule* L. (Wollenweber *et al.*, 1997a). Mainly flavonoid glycosides were reported from *Hieracium* spp. (Svehlíková *et al.*, 2002), and simple aglycones (ap, lut, and one unidentified flavone aglycone) were found in addition in some species from Montenegro (Petrovic *et al.*, 1999).

2.) *Hypochaeris maculata* L. yielded only apigenin and luteolin. No flavonoid aglycones could be detected in the leaf washes of *H. radicata* L. and *H. uniflora* Vill. In a recent phylogenetic study (Tremetsberger *et al*., 2005), *H. radicata* was found to be in a separate clade from *H. maculata*, which claded together with *H. uniflora*. This relationship is apparently not reflected by exudate flavonoid data. Glycosides of isoetin characterize species of *Hypochaeris* such as *H. radicata* and *H. uniflora*, and are mentioned also for *H. maculata* (Gluchoff-Fiasson *et al*., 1991). Free isoetin has so far not been found in exudates of these taxa.

3.) *Lapsana communis* L.: ap/-7-Me/-7,4′-diMe; lut/-3′-Me/-7,3′-diMe/-7,3′,4′-triMe; kae-3,7,4′-triMe; qu-3,7,3′-triMe/-3,7,3′,4′-tetraMe; lut-glycoside, chlorogenic acid. Caffeic acid, chlorogenic acid and 3 further derivates were earlier described from aerial parts (Fontanel *et al*., 1998).

4.) The exudate of *Sonchus arvensis* L. contained only lut and traces of qu-3′-Me. Flavonoid aglycones (ap-4′-Me, kae, lut/-3′-Me, qu-3′-Me) have been reported from the ethyl acetate extract of a Chinese accession (Qu *et al*., 1996).

Flavonoid aglycones of Astereae species

Species of *Grindelia*, *Gutierrezia*, *Hazardia*, *Nardophyllum*, and *Olearia* have been analyzed from this large tribe. *Grindelia* species are known

for their resinous nature. The 55 known species, being annual or perennial herbs, occur in North and South America. A similar geographic distribution characterizes the 27 herbal or shrubby species of *Gutierrezia*. *Hazardia* comprises 13 shrubby species of Southwest United States and North Mexico (Bremer, 1994). *Tonestus* is a segregate from the South American genus *Haplopappus*, with 8 recognized species occurring in North America (Nesom and Morgan, 1990), whereas *Haplopappus* s. str. is a South American genus consisting of 70 species (Bremer, 1994). *Nardophyllum* is a rather small genus of 10 South American species, being shrubs, sometimes spiny (Nesom, 1993). *Olearia* contains some 130 species, shrubs and trees, with a distribution centred in Australia and New Zealand (Bremer, 1994). This genus apparently is of polyphyletic origin, according to molecular systematic studies (Cross *et al*., 2002).

1.) *Grindelia chiloensis* (Cornell.) Cabr. yielded only kae-3-Me and kae-3,4′-diMe. The presence of kae-3-Me is in accordance with earlier reports on flavonoids from aerial parts (Ruiz *et al*., 1981). This shrubby species contains large amounts of resins, and it is currently under investigation as a possible resin crop (Wassner and Ravetta, 2005). The majority of resin components are of terpenoid nature, with some unidentified flavonoids mentioned (Zavala and Ravetta, 2002).

2.) *Grindelia robusta* Nutt.: Previous studies on cultivated material showed the presence of kae-3-Me/-3,4′-diMe; 6-OH-kae-3,6-diMe/-3,6,7-triMe; qu-3,3′-diMe/-3,7,3′-triMe/-3,3′,4′-triMe; queg-3,6,4′-triMe/-3,6,7,3′-tetraMe (Timmermann *et al.*, 1994). A new accession from cultivation in the Botanic Garden (BG-TUD) yielded a somewhat different profile, consisting of kae-3,7-diMe/-3,4′-diMe/-3,7,4′-triMe; 6-OH-kae-3,6,7-triMe/-3,6,4′-triMe; qu-3,4′-diMe/-3,7,3′-triMe/-3,7,4′-triMe/-3,7,3′,4-tetraMe. Similar trends have been observed for *Grindelia glutinosa* (Cav.) Dunal., yielding complex 6-OMe derivatives of kaempferol as exudate constituents (Timmermann *et al*., 1994). It appears that both species are closely related, as some authors assign only subspecies status to them (Timmermann *et al*., 1994). Further species studied for exudate flavonoids include *G. tenella* and *G. squarrosa* (Wollenweber *et al*., 1989) and *G. nana* var. *integrifolia* Nutt. (Wollenweber *et al*., 1997b). No 6-substituted flavonoids were found in the exudates of *G. tenella;* similarly, resin of *G. camporum* Greene contained only ap-4-Me, qu/-3,3′-diMe and kae-3,7-diMe (Hoffmann *et al*., 1984). Thus, flavonoid diversification could be of some systematic significance in this genus.

3.) *Gutierrezia resinosa* (Hook et Arn.) S. F. Blake: Two different accessions from Chile exhibited infraspecific differentiation as indicated below:

Accession a: qu-3-Me/-3,7-diMe; queg-3,6,7-triMe; goss-3,8-diMe/-3,7,8-triMe; 5,3′,4′-triOH-3,6,7,8-tetraOMe-flavone; 5,4′-diOH-3,6,7,8,3′-pentaOMe.

Accession b: qu-3-Me; queg-3,6-diMe/-3,7-diMe; goss-3,7,8-tri-OMe; 5,3′,4′-triOH-3,6,7,8-tetraOMe; 5,4′-diOH-3,6,7,8,3′-pentaOMe-flavone.

Earlier, literature data revealed the presence of 5,3′,4′-triOH-3,6,7,8-tetraOMe and of 5,4′-diOH-3,6,7,8,3′-pentaOMe (Bittner *et al*., 1983; Hoeneisen and Silva, 1986). Exudate flavonoids have been reported from *G. sarothrae* (Pursh) Britt. (Hradetzky *et al*., 1987); in the same paper, the occurrence of flavonoid aglycones in extracts of *G. grandis* and of *G. microcephala* is commented. Similar substitution trends have been observed in all species studied so far, as is also evident from results on *G. wrightii* (Fang *et al*., 1986).

4.) *Haplopappus glutinosus* Cass. ex DC.: ap; scut-6-Me/-6,4′-diMe; (kae-3-Me/-3,4′-diMe); 6-OH-kae-3,6-diMe/-3,6,4′-triMe; queg-3,6,3′-triMe. In contrast to other *Haplopappus* spp. (Valant-Vetschera and Wollenweber, 2004), there is a strong tendency towards formation of 6-methoxylated flavones and flavonols. So far, only flavonoid glycosides have been reported from this taxon (Marambio and Silva, 1996). A relatively poor profile (kae-3-Me; qu-3-Me/-3,3′-diMe; eriod-7-Me) characterizes *H. pectinatus* Phil., a species that is postulated to hybridize with *Grindelia chiloensis* (Bartoli and Tortosa, 1998), with a similarly incomplex flavonoid aglycone profile. It would be interesting to check further possible hybrids for their exudate flavonoid composition.

5.) *Hazardia berberidis* Greene: (kae-3,7-diMe)/-3,4′-diMe/-7,4′-diMe/-3,7,4′triMe; qu/-3-Me/-7-Me/-3′-Me/-3,7-diMe/-3,3′-diMe/-3,4′-diMe/-7,3′-diMe/-3,7,4′-triMe/-3,3′,4′-triMe/-7,3′,4′-triMe/-3,7,3′,4′-tetraMe.

6.) *Hazardia ferrisiae* (S. F. Blake) W. D. Clark: kae-3-Me/-7-Me/-3,4′-diMe.

7.) *Hazardia orcuttii* Greene: kae-3-Me/-3,7-diMe; qu/-3-Me/-3,3′-diMe; goss-3,8-diMe. In these *Hazardia* species, flavonols are the dominat-

ing exudate compounds, with only one species accumulating 8-substituted flavonols, whereas 6-substituted flavones and flavonols, along with eriodictyol derivatives, were found in exudates of *H. squarrosa* Greene var. *grindelioides* (DC.) W. D. Clark (Clark and Wollenweber, 1985). Taken all species analyzed so far together, the resulting accumulation trends are quite complex and diversified.

8.) *Nardophyllum scoparium* Phil.: qu-3-Me; queg-3,6-diMe/-3,6,7-triMe/-3,6,4′-triMe; (5,7,3′,4′-tetraOH-3,6,8-triOMe-flavone;) 5,7,3′-triOH-3,6,8,4′-tetraOMe-flavone. This appears to be the first report on flavonoids compounds in this genus, which so far has only been studied for diterpenes (*e.g.* Zdero *et al*., 1990). Controversies as to the taxonomic position have been successfully resolved (Nesom, 1993).

9.) *Olearia glutinosa* Benth: ap; kae-3-Me/-3,4′-diMe; qu-3-Me/-7-Me/-3′-Me/-7,4′-diMe/-3,4′-diMe/-3,7,3′-triMe/-3,7,3′,4′-tetraMe; eriod-7-Me (/-7,3′-diMe).

10.) *Olearia ramulosa* Benth.: ap/-7-Me; lut/-7-Me/-3′-Me/-4′-Me/-7,3′-diMe; kae; qu/-3′-Me. The profiles of both species are quite different: flavanones occur additionally in exudates of *O. glandulosa*, while flavones predominate in those of *O. ramulosa*. In a recent phylogenetic study, both *Olearia* species were placed in different clades (Cross *et al*., 2002), and it would be interesting to test more species of those clades for eventual exudate flavonoid diversification. Leaf extracts of *Olearia muelleri* (Sonder) Benth., coming in the same clade as *O. ramulosa* (Cross *et al*., 2002), contained queg-3,6,4′-triMe and queg-3,6-diMe (Jefferies *et al*., 1974), while those of *O. paniculata* (J. R. and G. Forst) Druce of the second clade contained the flavone scut-6,4′-diMe (Chivers *et al*., 1966). It is assumed that these compounds are also exudate constituents, and accumulation trends may prove to be group-specific.

11.) *Tonestus lyallii* (A. Gray) A. Nelson: (ap)/-7-Me/-7,4′-diMe; lut/-3′-Me/-7-Me; 6-OH-lut-6-Me/-6,4′-diMe/-6,7,3′-triMe; kae/-7-Me; qu/-3′-Me/-7,3′-diMe. This aglycone profile corresponds to trends observed in this tribe, and it is not specific enough to separate this species clearly from those of *Haplopappus.*

Flavonoid aglycones of Senecioneae species

Senecio is quite a large genus of about 1250 species of world-wide distribution, and with a range of well differentiated growth forms. The genus *Euryops* comprises some 97 species, mostly shrubs or subshrubs, with occurrence in South, tropical- and Northeast Africa, Arabia (Bremer, 1994).

1.) *Senecio murinus* Phil., a Chilean species, contained kae-3-Me/-3,7-diMe/-3,7,4′-triMe; qu-3,7-diMe in its exudate. The exudate of *S. viscosa* L. contained some simple flavone and flavonol methyl ethers (Wollenweber *et al*., 1997a), corresponding in substitution patterns to the new results. It is a pity that only so few species could so far be analyzed for exudate compounds.

2.) *Euryops acraeus* M. D. Hend. exhibited a poor profile, consisting of kae and qu-3′-Me only. Altogether, *Senecioneae* are apparently not very productive in terms of exudate flavonoids, and structures appear to be quite simple in terms of substitution patterns.

Flavonoid aglycones of Helenieae species

Only one species of *Eriophyllum* was studied from this tribe. Species of this genus are either subshrubs or herbs, and 11 species are distributed in the West USA, Northeast Mexico and Southwest Canada (Bremer, 1994). *Eriophyllum lanatum* (Pursh.) Forbes yielded several exudate flavonoids: ap; scut-6,4′-diMe; lut; 6-OH-lut-6-Me/-6,3′,4′-triMe; queg-3,6,3′,4′-tetraMe. Earlier, queg-6-Me/-3,6-diMe/-6,3′-diMe/-3,6,4′-triMe were isolated from *E. confertifolium* (DC.) A. Gray, while *E. staechadifolium* Lag. yielded qu/-3-Me/-3′-Me/-3,3′-diMe; queg-3,6-diMe/-3,6,3′-triMe (Wollenweber *et al.,* 1997b). Apart from these results, no further flavonoid data are available on this genus.

Flavonoid aglycones of Heliantheae species

Species of *Iva*, *Sigesbeckia*, *Silphium*, *Xanthium, Zinnia* and *Balsamorhiza* were studied here. *Iva* consists of 15 North American species, being herbs or shrubs. *Sigesbeckia* is a small taxon with 3 annual species from tropical Africa and Asia. *Silphium* comprises 23 species (perennial herbs) distributed in the United States. *Xanthium* with its 3 sometimes spiny species is widespread in warm parts of the world. The 22 species of *Zinnia* grow in the South of North America, Mexico, Central and South America as shrubs or herbs, and *Balsamorhiza* consists of 14 species, occurring in the West of North America and Mexico as perennial herbs (Bremer, 1994).

1.) *Iva xanthifolia* Nutt.: 5,7,4′-OH-6,8-OMe-flavone (desmethoxysudachitin); 5,4′-OH-6,7,8-OMe-flavone (xanthomicrol); 5,7-OH-6,8,4′-OMe-flavone (nevadensin). The generic concept of *Iva* has been recently revised, and it was suggested to treat *I. xanthifolia* in the segregate genus *Cyclachaena* (Miao *et al*., 1995).

2.) *Sigesbeckia flocculosa* L'Her.: qu/-3-Me/-3,7-diMe/-3,7,4′-triMe; (eriod;) qu-3-glucoside; chlorogenic acid. This Peruvian species differs from *S. jorullensis* Kunth, of which que-8-Me was earlier described, and from *S. orientalis*, which had yielded qu-5-Me (Wollenweber *et al*., 1989). *Sigesbeckia jorullensis* was analyzed lately regarding the morphology of glandular hairs and their essential oil production (Heinrich *et al*., 2002), but flavonoids have not been specified in this publication.

3.) *Xanthium strumarium* L.: 6-OH-kae-6-Me/-3,6-diMe; queg-3,6-diMe/-3,6,3′-triMe. Earlier, only small amounts of exudate were obtained from another accession, yielding 6-OH-kae-6-Me and queg-3,6-diMe (Wollenweber *et al*., 1997a).

4.) *Zinnia elegans* Jacq.: ap/-7-Me/-4′-Me/-7,4′-diMe; lut/-7-Me. Earlier, *Z. acerosa* (DC.) A. Gray was found to accumulate a series of 8-OMe flavone derivatives in its exudate (Wollenweber *et al*., 1997b).

5.) *Silphium laciniatum* L.: (kae; qu-3-Me/-3′-Me;) 6-OH-kae-6-Me/-3,6-diMe; quercetagetin-6-Me/-3,6-diMe/-6,3′-diMe/-3,6,3′-triMe; kae-3-glucoside; qu-3-glucoside; and eriod-3′-Me-6-C5.

6.) *Silphium terebinthinaceum* Jacq. yielded kae-3-glucoside; qu-3-rhamnoside; qu-3-glucoside and qu-3-rhamnoglucoside from the leaf washes. This species thus affords an example of lack of aglycones in the exudate, which is quite uncommon among the Asteraceae. Limited literature exists on flavonoids of this genus, mentioning mainly the occurrence of flavonol glycosides in extracts (*e.g.* El-Sayed *et al.*, 2002).

7.) *Balsamorhiza sagittata* (Pursh.) Nutt.: Minor infraspecific variability was noted between several accessions studied. Major compounds in all accessions were queg-6-Me and 6-OH-kae-6-Me, accompanied by queg-6,3′-diMe and 6-OH-kae-6,4′-diMe and qu. Variation was noted for queg-3,6,3′-triMe as well as kae and kae-4′-Me.

8.) *Balsamorhiza macrophylla* Nutt. yielded qu; qu-3-Me; qu-3′-Me; (qu-3,3′-diMe;) qu-3,3′,4′-triMe; queg-3,6,3′,4′-tetraMe. The accumulation trends of both taxa are in line with earlier publications (Bohm and Choy, 1987; Bohm *et al*., 1989), except for the report on qu-4′-Me (Robson and McCormick, 1988), which was not found now in the exudates. Also, 6-OH-kae-7-Me and queg-7-Me reported from *B. deltoidea* could not be found in any of the samples studied now. In terms of accumulation trends, *Balsamorhiza* differs from the closely related *Silphium* (Clevinger and Panero, 2000) by a more complex exudate profile.

The 7-methyl ethers of 6-OH-kae and of queg were reported earlier as flavonoid aglycones from *B. deltoidea* (Bohm and Coy, 1987). Referring to this paper, 6-OH-kae-7-Me was later reported as "a single major flavonoid from leaf exudate of *B. sagittata*" (Bohm *et al*., 1989). However, synthesis revealed that both structures were not correct (Tominaga and Horie, 1993). As a matter of fact, our thorough search for these two flavonols in all our samples of *B. sagittata* (as well as in samples of *B. deltoidea*), using snythetic markers, proved their absence.

Chemodiversity at the tribal level

In terms of accumulation tendencies, it appears that the tribes of the Cichorioideae have a less complex aglycone composition, as far as oxygenation patterns are concerned. Trends in the *Mutisieae* include formation of mainly flavonol methyl ethers except for those with 6- or 8-methoxylation. Flavones are not so common, but flavanones have been found occasionally. Trends in *Cardueae* as based upon a single genus only indicate a relative poorness in terms of chemical diversity. Earlier, 6-substituted flavones and flavonols had been found in *Centaurea* exudates, but *Cirsium* yielded only relatively simple flavones and flavonols (Wollenweber and Valant-Vetschera, 1996). *Lactuceae* trends are almost identical to the *Mutisieae* trends. Genera of the *Lactuceae* appear to accumulate rarely exudate flavonoids. In the positive cases, mostly rather simple flavone or flavonol derivatives were found so far. Similar results have been obtained with new species and accessions studied now. *Hieracium* is remarkable as it also yielded flavanones. The poorest profile was observed in *Sonchus arvensis*. Despite the low yield, structures are sometimes quite diversified. These data are well in accordance with earlier observations on flavonoid aglycone diversification in genera of the Cichorioideae (Wollenweber and Valant-Vetschera, 1996).

Astereae and *Heliantheae* exhibit the largest degree of complexity. Within *Astereae*, quercetagetin and/or gossypetin methyl ethers have been frequently found in the exudates of some species of *Grindelia*, *Gutierrezia*, *Hazardia* and *Nardophyllum*. Especially some *Astereae* are known for their high resin content (*e.g.* *Grindelia*). Both tribes were earlier observed to have quite some diversity in their oxygenation patterns (Wollenweber and Valant-Vetschera, 1996). The presence of flavonol glycosides in leaf washes of some *Heliantheae* is remarkable. The *Helenieae* come close to the *Heliantheae* in the complexity of their exudates as had been exemplified earlier (Wollenweber and Valant-Vetschera, 1996).

At present, it looks as if the tribes of the Asteroideae are much more complex in their exudate flavonoid chemistry as compared to the Cichorioideae. This is also true for genera of other tribes that had been studied before (Valant-Vetschera and Wollenweber, 2004; Wollenweber *et al*., 1997a, b, 2005). The only exception is represented by the *Senecioneae*, which showed little complexity in the exudates. It has to be mentioned that from this group very little data are available, and that especially the large genus *Senecio* would need more investigations. Correlation of exudate flavonoid accumulation to the existence of glandular structures, which excrete compounds on the leaf surfaces, and the preferences for xeric or alpine habitats is again confirmed, as had been indicated earlier (*e.g.* Wollenweber and Valant-Vetschera, 1996).

Acknowledgements

Thanks are due to Bruce Bohm, Vancouver (Canada), Dennis Clark, Tempe, AZ (USA), H. Groh, Münster (Germany), P. López, Concepcion (Chile), Andrew Rozefelds, Hobart (Tasmania), Tod Stuessy, Vienna (Austria) and George Yatskievych, St. Louis, MO (USA) as well as to staff members of the Botanical Garden at Darmstadt for plant material. E. W. is also grateful to T. Horie, Tokushima (Japan) for a gift of synthetic 6-OH-kae-7-Me and queg-7-Me and to J. N. Roitman, Albany, CA (USA) for structural identification of the 6-*C*-prenyl derivative of eriodictyol-3-methyl ether. The skilful technical assistance of Marion Doerr, Darmstadt (Germany) is also gratefully acknowledged.

Bartoli A. and Tortosa R. D. (1998), Intermediacy between *Grindelia chiloensis* and *Haplopappus pectinatus* (Astereae, Asteraceae). Taxon **47**, 337–345.

Belenovskaja L. (1996), *Artemisia*: Compositae-Systematics, Proceedings of the International Compositae Conference, Kew 1994, Vol. I (Hind D. J. N. and Beentje H. J., eds.). Royal Botanic Gardens, Kew, pp. 253–259.

Bittner M., Silva M., Vargas J., and Bohlmann F. (1983), Biologically active flavones from *Gutierrezia resinosa*. Phytochemistry **22**, 1523–1524.

Bittner M., Jakupovic J., Bohlmann F., and Silva M. (1989), Coumarins and guaianolides from further Chilean representatives of the subtribe *Nassauviinae*. Phytochemistry **28**, 2867–2868.

Bohm B. A. and Choy J. B. (1987), Flavonoids of *Balsamorhiza deltoidea* and *Wyethia mollis*. Biochem. Syst. Ecol. **15**, 541–543.

Bohm B. A., Choy J. B., and Lee A. Y.-M. (1989), Flavonoids of *Balsamorhiza* and *Wyethia*. Phytochemistry **28**, 501–503.

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

Chivers H., Corbett R. E., and Mitchell R. E. M. (1966), Extractives from the leaves of *Olearia paniculata*. J. Chem. Soc. **20 C**, 1814–1816.

Clark W. D. and Wollenweber E. (1985), Flavonoid aglycones in the resin of *Hazardia squarrosa* var. *grindelioides*. Phytochemistry **24**, 1122–1123.

Clevinger J. A. and Panero J. L. (2000), Phylogenetic analysis of *Silphium* and subtribe *Engelmanniinae* (Asteraceae: Heliantheae) based on ITS and ETS sequence data. Am. J. Bot. **87**, 565–572.

Cross E. W., Quinn C. J., and Wagstaff S. J. (2002), Molecular evidence for the polyphyly of *Olearia* (Astereae: Asteraceae). Plant Syst. Evol. **235**, 99–120.

Delporte C., Backhouse N., Erazo S., Negrete R., Vidal P., Silva X., López-Pérez J. L., San Feliciano A., and Muñoz O. (2005), Analgesic-antiinflammatory properties of *Proustia pyrifolia*. J. Ethnopharmacol. **99**, 119–124.

El-Sayed N. H., Wojcinska M., Drost-Karbowska K., Matlawska I., Williams J., and Mabry T. J. (2002), Kaempferol triosides from *Silphium perfoliatum*. Phytochemistry **60**, 835–838.

Emerenciano V. P., Militao J. S. L. T., Campos C. C., Romoff P., Kaplan M. A. C., Zambon M., and Brant A. J. C. (2001), Flavonoids as chemotoxonomic markers for Asteraceae. Biochem. Syst. Ecol. **29**, 947–957.

Faini F., Torres R., and Castillo M. (1984), Costunolide and flavonols from *Gochnatia foliosa* var. *fascicularis*. J. Nat. Prod. **47**, 552.

Fang N., Yu. S., and Mabry T. J. (1986), Flavonoids from *Gutierrezia wrightii*. J. Nat. Prod. **49**, 738–740.

Fontanel D., Galtier C., Viel C., and Gueiffler A. (1998), Caffeoyl quinic and tartaric acids and flavonoids from *Lapsana communis* L. subsp. *communis* (Asteraceae). Z. Naturforsch. **53c**, 1090–1092.

Freire S. E., Katinas L., and Sancho G. (2002), *Gochnatia* (Asteraceae, Mutisieae) and the *Gochnatia* complex: Taxonomic implications from morphology. Ann. Missouri Bot. Gard. **89**, 524–550.

Gluchoff-Fiasson K., Favre-Bonvin J., and Fiasson J. L. (1991), Glycosides and acylated glycosides of isoetin from European species of *Hypchaeris*. Phytochemistry **30**, 1673–1675.

Heinrich G., Pfeifhofer H. W., Stabentheiner E., and Sawidis T. (2002), Glandular hairs of *Sigesbeckia jorullensis* Kunth (Asteraceae): morphology, histochemistry and composition of essential oil. Ann. Bot. **89**, 459–469.

Hoeneisen M. and Silva M. (1986), Quimica actividad biologica de algunas Compositae Chilenas. Rev. Latinoam. Quim. **17**, 19–24.

Hoffmann J. J., Kingsolver B. E., McLaughlin S. P., and Timmermann B. (1984), Production of resins by arid-adapted Astereae. In: Recent Advances in Phytochemistry, Vol. 18 (Timmermann B. N., Steelink C., and Loews R., eds.). Plenum Publ. Co., New York, pp. 251–271.

Hradetzky D., Wollenweber E., and Roitman J. R. (1987), Flavonoids from the leaf resin of snakeweed, *Gutierrezia sarothrae*. Z. Naturforsch. **42c**, 73–76.

Jefferies P. R., Knox J. R., Price K. R., and Scaf B. (1974), Constituents of the tumor-inhibitory extract of *Olearia muelleri*. Austr. J. Chem. **27**, 221–225.

Marambio O. and Silva M. (1996), Compuestos fenolicos y triterpenos aislados desde *Haplopappus velutinus* Remy y *H. glutinosus* Cass. Bol. Soc. Chil. Quim. **41**, 199–200.

Miao B., Turner B. L., and Mabry T. J. (1995), Chloroplast DNA variations in sect. *Cyclachaena* of *Iva* (Asteraceae). Am. J. Bot. **82**, 919–923.

Nesom G. L. (1993), Taxonomic status of *Nardophyllum scoparium* (Asteraceae-Astereae) with observations on the definition of *Nardophyllum*. Phytologia **75**, 358–365.

Nesom G. L. and Morgan D. R. (1990), Reinstatement of *Tonestus* (Asteraceae:Astereae). Phytologia **68**, 184–180.

Petrovic S. D., Löscher R., Gorunovic M. S., and Merfort I. (1999), Flavonoid and phenolic patterns in seven *Hieracium* species. Biochem. Syst. Ecol. **27**, 651–656.

Qu G., Li X., and Liu J. (1996), Studies on flavonol glycosides of *Sonchus arvensis* L. Zhongguo Zhongyao Zazhi **21**, 292–294.

Robson K. A. and McCormick S. P. (1988), 6-Methoxyflavonoids from *Balsamorhiza* section *Artorhiza*. Biochem. Syst. Ecol. **16**, 411–412.

Ruiz S. O., Guerreiro E., and Giordano O. S. (1981), Flavonoids in three species of *Grindelia*. An. Asoc. Quim. Argentina **69**, 293–295.

Sacilotto A. C. B. C., Vichnewski W., and Herz W. (1997), *Ent*-Kaurene diterpenes from *Gochnatia polymorpha* var. *polymorpha*. Phytochemistry **44**, 659–661.

Svehlíková V., Mráz P., Piacente S., and Marhold K. (2002), Chemotaxonomic significance of flavonoids and phenolic acids in the *Hieracium rohacsense* group (*Hieracium* sect. *Alpina*; Lactuceae, Compositae). Biochem. Syst. Ecol. **30**, 1037–1049.

Timmermann B., Wollenweber E., Dörr M., Armbruster S., Valant-Vetschera K. M., and Fuentes E. R. (1994), External flavonoids in two *Grindelia* species. Z. Naturforsch. **49c**, 395–397.

Tominaga H. and Horie T. (1993), Studies of the selective *O*-alkylation 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.

Tremetsberger K., Weiss-Schneeweiss H., Stuessy T., Samuel R., Kadelc G., Ortiz M. A., and Talavera S. (2005), Nuclear ribosomal DNA and karyotypes indicate a NW African origin of South American *Hypochaeris* (Asteraceae, Cichorieae). Mol. Phylogenet. Evol. **35**, 102–116.

Valant-Vetschera K. M. and Wollenweber E. (2004), Chemodiversity of exudate flavonoids in some *Haplopappus* species from Chile. Polyphenol Communications 2004, pp. 707–708 (XXII International Conference on Polyphenols, 25.–28. August 2004, Helsinki, Finland).

Wassner D. F. and Ravetta D. A. (2005), Temperature effect on leaf properties, resin content, and composition in *Grindelia chiloensis* (Asteraceae). Ind. Crops Prod. **21**, 155–163.

Wollenweber E. (1984), Exkret-Flavonoide bei *Hieracium intybaceum* (Asteraceae), einem alpinen Habichtskraut. Z. Naturforsch. **39c**, 833–834.

Wollenweber E. and Valant-Vetschera K. M. (1996), New results with exudate flavonoids in Compositae. In: Compositae-Systematics, Proceedings of the International Compositae Conference, Kew 1994, Vol. I (Hind D. J. N. and Beentje H. J., eds.). Royal Botanic Gardens, Kew, pp. 169–185.

Wollenweber E., Mann K., and Hochwart S. (1989), Exudate flavonoids in miscellaneous Asteraceae. Phytochem. Bull. **21**, 19–23.

Wollenweber E., Dörr M., Fritz H., and Valant-Vetschera K. M. (1997a), Exudate flavonoids in several Cichorioideae and Asteroideae (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., Christ M., Dunstan R. H., Roitman J. N., and Stevens J. F. (2005), Exudate flavonoids in some Gnaphalieae and Inuleae (Asteraceae). Z. Naturforsch. **60c**, 671–678.

Ybarra M. I., César A. N., Catalán J. D., and Herz W. (1994), Two homofarnesane derivatives and other constituents from *Gochnatia glutinosa*. Phytochemistry **35**, 1585–1587.

Zavala J. A. and Ravetta D. A. (2002), The effect of solar UV-B radiation on terpenes and biomass production in *Grindelia chiloensis* (Asteraceae), a woody perennial of Patagonia. Plant Ecol. **161**, 185–191.

Zdero C., Bohlmann F., and Niemeyer H. M. (1990), Diterpenes from *Nardophyllum lanatum*. Phytochemistry **29**, 1227–1230.

Zemtsova G. N. and Molchanova L. P. (1979), Flavonoids and triterpenoids of *Xeranthemum annuum*. Khim. Prir. Soed. **6**, 856–857.