Flavonoid Aglycones from the Leaf Surfaces of Some *Artemisia* spp. (Compositae–Anthemideae)

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

[a] Institut für Botanik der Universität Wien, Rennweg 14, A-1030 Wien, Austria
[b] Institut für Botanik der TH, Schnittspahnstrasse 3, D-64287 Darmstadt, Bundesrepublik Deutschland

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Several species of the large genus *Artemisia* have been analysed for the flavonoid aglycone composition of their leaf exudates. These species belong to different taxonomic groups within *Artemisia*. Flavone and flavonol aglycones were found as the major flavonoid constituents of the leaf exudates. Many of these compounds were 6-methoxylated, with additional substitutions at the 7-, 3′- and 4′-position of the molecule. In addition, 7,4′-substituted apigenin and luteolin derivatives and rarely also coumarin derivatives were encountered in some species. The observed substitution trends are in accordance with literature data. Taxonomic and ecological aspects are briefly discussed.

Introduction

The genus *Artemisia* (Compositae–Anthemideae) comprises more than 500 species, including the sometimes separated genus *Seriphidium* (Bremer and Humphries, 1993). Many *Artemisia* species occur in dry habitats or in the alpine region, mainly in Eurasia and North America. Only very few species are known from South America or Southern Africa.

As could be shown in previous publications, species of this genus may be good sources for exudate flavonoid aglycones (Wollenweber *et al.*, 1989; Wollenweber and Rustaiyan, 1991; Wollenweber *et al.*, 1992). These species produce a wide array of flavones and flavonols, mainly based upon 6-substituted derivatives. Occasionally, also rarer compounds such as dihydroflavonols have been reported as exudate constituents (Wollenweber *et al.*, 1989, and references cited therein). In continuation of our studies on the distribution of exudate flavonoids, we wish to report the flavonoid composition of further *Artemisia* species of different systematic groups.

Materials and Methods

Aerial parts including inflorescences were collected either from cultivated material of the Botanical Garden, Univ. of Vienna or from natural sites. The list of species analysed is presented along with Table I. Herbarium specimens are deposited in the herbaria of the collectors' institutions (WU, MO). Air-dried plant material was briefly rinsed with acetone at room temperature to dissolve the exudate material. After evaporation of the solvent, the residue was chromatographed over Sephadex LH-20, eluted with methanol, to separate the flavonoids from the dominating terpenoids. Individual flavonoids were identified in relevant fractions by co-chromatography with authentic samples available in E.W.'s lab. Fractions were monitored on TLC and comparisons with markers were performed on silica with toluene/MeCOEt 9:1 and with toluene/dioxane/glacial acetic acid 18:5:1, and on polyamide with toluene/petrol$_{100-140}$/MeCOEt/MeOH 12:6:2:1 and toluene/dioxane/MeOH 8:1:1. Chromatograms were viewed under UV_{366} before and after spraying with "Naturstoffreagenz A" (NA). For additional structural confirmation, UV-spectra and mass spectra were recorded when necessary.

Results

The *Artemisia* species analysed here belong to different systematic groups. Their arrangement in Table I follows principally the taxonomic concept of Poljakov (1961) with some modifications. Thus, *A. iwayomogi* Kitam., *A. santolinifolia* Turcz. ex

Reprint requests to K. M. Valant-Vetschera.
Telefax: 1-79794131.

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Table I. Distribution of exudate aglycones in *Artemisia* spp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Apigenin														●
-7-Me	●	●												●
-4′-Me	●	●		●										●
-7,4′-diMe														●
Luteolin		●	●	●	●	●			●	●		●	●	●
-3′-Me		●		●	●									●
-4′-Me		●		●										
-7-Me	●	●		●										
-7,3′-Me	●	●												
-7,4′-Me		●												
Kaempferol														
-3-Me						●								
Quercetin														●
-3-Me					●									●
-3,3′-Me					●									
-7,3′-Me			●											
Scutellarein						●		●	●	●				
-6-Me						●	●							
-6,7-Me														●
-6,4′-diMe														
6-Hydroxyluteolin													●	
-6-Me	●	●		●	●	●	●	●	●	●				
-6,7-Me				●		●								
-6,3′-Me	●					●	●	●	●	●	●			
-6,7,3′-Me						●								
-6,7,4′-Me						●								
-6,3′,4′-Me						●	●	●	●			●		●
-6,7,3′,4′-Me						●	●							
6-OH-Kaempferol														
-3,6-Me												●	●	
-3,6,4′-Me												●		
Quercetagetin														
-3,6-Me			●	●				●		●	●	●	●	●
-6,3′-Me								●			●			
-3,6,3′-Me			●					●	●	●	●	●		
-3,6,4′-Me				●								●		
-3,6,3′,4′-Me								●	●		●			
-3,6,7-Me			●	●	●	●							●	
-3,6,7,3′-Me			●											
-3,6,7,4′-Me				●										
Coumarins		×			Sco								×	
Phloroetophenone														
-diMe														●

List of analysed *Artemisia* species:

1 *A. iwayomogi* Kitam., cult. Bot. Garden, Univ. Vienna (AR-1267), Japan, Hokkaido, Shribershi.
2 *A. iwayomogi* Kitam., cult. Bot. Garden, Univ. Vienna (AR-807), Japan, Hokkaido, Prov. Hiyama.
3 *A. santolinifolia* Turcz. ex Besser, cult. Bot. Garden, Univ. Vienna, (AR-1126). Tadschikistan.
4 *A. molinieri* Quezel, cult. Bot. Garden, Univ. Vienna, (AR-300). France,, Flassans-sur-Issole.
5 *A. alba* Turra, Makedonia, Galicica mountains. coll. K. Valant-Vetschera, 1985.
6 *A. austria* Jq., Austria, Burgenland, Neusiedl. coll. K. Valant-Vetschera, 1992.
7 *A. austria* Jq., cult. Bot. Garden, Univ. Vienna (AR 1212). Moldova, Kishinev.
8 *A. mutellina* Vill. cult. Pietraporzi, Cuneo (Italy), coll. Appendino.
9 *A. schmidtiana* Maxim., cult. Bot. Garden, Univ. Vienna (AR-975). Japan, Sapporo.
10 *A. douglasiana* Besser, California, Humboldt County, coll. GY 88–179, MO.
11 *A. ludoviciana* Nutt., cult. Bot. Garden, Univ. Vienna (AR-947). Kanada, Osoyoos.
12 *A. ludoviciana* Nutt. subsp. *incompta* (Nutt.) Keck, cult. Bot. Garden, Univ. Vienna (AR-926). Utah, Clear Creek Canyon.
13 *A. tridentata* Nutt., California, San Bernadino County, GY-90–58, MO.
14 *A. diffusa* Krasch. ex Poljak., Iran, coll. A. Rustayian.

Coumarins: Sco = scopoletin; × indicates unidentified coumarins.

Bess. and *A. molinieri* Quezel belong to sect. *Abrotanum*, whereas *A. austriaca* Jacq., *A. mutellina* Vill. and *A. schmidtiana* Maxim. belong to sect. *Absinthium*. The taxonomic position of *A. alba* Turra is not yet clarified. According to Gutermann (1979), this species is rather related to the species group around *A. pontica* L., and thus has to be grouped within sect. *Abrotanum*. Sect. *Artemisia* houses *inter alia* the "*Vulgares*" group with *A. douglasiana* Bess. and *A. ludoviciana* Nutt.. By contrast, *A. tridentata* Nutt. and *A. diffusa* Krasch. ex Poljak. are grouped within sect. *Seriphidium*, which has recently been proposed as the genus *Seriphidium* (Bremer and Humphries, 1993). *A. tridentata* and related species, being morphologically different from the rest of this section, are sometimes considered to form the subgenus Tridentatae (Gutermann, 1979). The genus *Artemisia* incorporates also the sect. *Dracunculus*. In previous studies, species of this taxonomic group such as *A. glauca* Pall. ex Willd. yielded flavones, flavonols, flavanones and coumarins, and *A. campestris* L. yielded 6-hydroxyflavone methyl ethers as exudate constituents (Wollenweber *et al.*, 1989).

Considering the size and morphological differentiations in this large genus, chemical character variation may be expected among the flavonoids as has been already shown for several classes of compounds such as polyacetylenes, sesquiterpene lactones and lignans (Bohlmann *et al.*, 1973; Greger 1982; Seaman, 1982). Concerning exudate aglycone formation, several substitution trends become apparent (Table I). These substitution trends include the formation of flavones and flavonols with 7-, 3′-,4′- substitutions and of 6-substituted flavones and flavonols, carrying frequently additional methoxy groups in these and further positions. Especially the 6-substituted polymethoxyflavones and -flavonols appear to be a characteristic trend of *Artemisia* and of the tribe Anthemideae. Presently it appears that the corresponding 8-substituted derivatives are of restricted occurrence in this tribe and particularly in the genera *Achillea* and partly also in *Artemisia* (Wollenweber and Valant-Vetschera, 1995). In some species, also coumarins were detected as constituents of the exudates. This is not a new finding (Wollenweber *et al.*, 1989), and the presence of coumarins does not appear to be specific for taxonomic groups within the genus.

Within sect. *Abrotanum*, there is a strong tendency towards formation of flavones with 3′-, 4′- and 7-methoxygroups, as was observed for *A. iwayomogi* (No. 1, 2 in Table I) and *A. molinieri* (No. 4).The 6-substituted flavone derivatives are rarely accumulated in *A. iwayomogi*, but 6-substituted flavonols occurr in the exudates of *A. santolinifolia* (No. 3) and *A. molinieri* (No. 4). The closely related *A. gmelinii* Web. ex Stechm. yielded 7,4′-dimethyl apigenin, 6-hydroxyluteolin 6,3′-dimethyl ether and luteolin 7,3′-dimethyl ether in the aerial parts (Cemesova *et al.*, 1983), thus confirming our results. *Artemisia alba* (No. 5 in Table I) accumulates mainly simple flavone- and flavonol derivatives and only one polymethoxyflavonol. Scopoletin was identified as coumarin constituent of the exudate. In comparison to earlier studies, the observed differences indicate a relatively high degree of infraspecific variation (Wollenweber *et al.*, 1989). Altogether, the flavonoid profile is somewhat nondescript and thus does not facilitate the systematic position of this critical taxon. However, other chemical characters point to a closer relationship to the species group around *A. pontica*. The flavonoid substitution trends of *A. pontica*, of *A. afra* Jacquem. ex Willd. and of *A. abrotanum* L. are well in accordance with the patterns observed so far in this section (Wollenweber *et al.*, 1989). Taking possible infraspecific variation into account, the substitution trends of these exudate flavonoids might prove to be of taxonomic significance in this section.

The analysed species from sect. *Absinthium* are characterised by the formation of 6-substituted flavones and -flavonols in their exudates. Derivatives mainly of 6-hydroxyluteolin constitute the profile of *A. austriaca*. There was some variation noted in formation of single compounds (Table I). The profile of No. 6, collected in Austria, consists of more derivatives, including quercetagetin 3,6,7-trimethyl ether. By contrast, the cultivated material from Moldavia (No. 7 in Table I) exhibits a less diversified pattern. In search for the rare 5-OH-7,4′-diOMe-6-CMe flavone, which has been reported for a collection of *A. austriaca* (Adekenov *et al.*, 1987), several other collections from various locations have been analysed. These collections include material from France, Iran, Armenia, Romania (Transsilvania) and Poland. Despite the geographic dislocation of the populations studied,

hardly any infraspecific varitation was noted in these samples. Furthermore, the profiles consisted of the same compounds as reported from the bulk material (No. 6 in Table I). The C-methylether was not found in any of the samples studied. In all probability, this particular compound does not occur in the aerial parts of *A. austriaca*.

Additional formation of 6-substituted flavonols characterises *A. mutellina* (No. 8 in Table I) from alpine regions in Europe and *A. schmidtiana* Maxim. (No. 9 in Table I) from Japan. Both species exhibit a very similar flavonoid profile. The structural diversity within the 6-hydroxyflavones appears to be reduced, as compared to *A. austriaca*. 6-hydroxyluteolin 6,3′,4′-trimethyl ether has been reported as exudate constituent in *A. mutellina* (syn. *A. umbelliformis* Lam.; Cappelleti *et al.*, 1986), which could be confirmed by our studies. Other species of sect. *Absinthium* exhibited a strong tendency to formation of higher substituted 6-hydroxyflavonols (Wollenweber *et al.*, 1989). It would be interesting to study the substitution trends in more detail within this section.

Within sect. *Artemisia*, the species of the "*Vulgares*" group produce less essential oils, with a few exceptions such as *A. douglasiana* or *A. ludoviciana*. Hence, it is not surprising that these species yielded also exudate flavonoids. The aglycone profile of the North-American *A. douglasiana* (No. 10 in Table I) consists mainly of 6-hydroxyflavones and -flavonols with a relatively low degree of methylation. In *A. ludoviciana*, 6-hydroxyflavonols predominate in the exudates (No. 11, 12 in Table I). This species is divided into several subspecies; their relations and evolutionary development have been the studied in detail (Estes, 1969). The botanical complexity is reflected also by the encountered aglycone variation. Aerial parts of another collection yielded even more flavonoid compounds, including tricin and other 5′-substituted derivatives (Liu and Mabry, 1982; Ruiz-Canino *et al.*, 1993). In addition, also 8-substituted flavonols were reported to occur in some populations (Dominguez and Cardenas, 1975; Liu and Mabry, 1982). Such compounds have not been detected so far during our studies on exudate flavonoids in *Artemisia*. Maybe some of these compounds are rather accumulated in the inflorescences than in the exudate of leaves and stems.

Finally, two species of the large sect. *Seriphidium* were analysed here. *A. tridentata* is native to Northern America, covering wast areas of land. Its taxonomy is quite complicated due to autopolyploidy (McArthur *et al.*, 1981). Hybridisation between subspecies has also been observed (Freeman *et al.*, 1991). The flavonoid exudate of one collection (No. 13 in Table I) contains mainyl 6-hydroxyluteolin- and quercetagetin derivatives. Earlier, aerial parts of another collection were reported to accumulate 6-hydroxykaempferol 3,6-dimethyl ether and 6-hydroxyluteolin 6-methyl ether (Brown *et al.*, 1975). Another population afforded quercetagetin and 6-hydroxykaempferol 3,6,7-trimethyl ethers along with quercetagetin 3,6-dimethyl ether (Rodriguez *et al.*, 1972). In comparison to other *Artemisia* species, the flavonoid profile so far known appears to be relatively simple in terms of substitution trends.

Artemisia diffusa, by contrast, shows a more diversified exudate profile. One collection (No. 14 in Table I) yielded some simple apigenin- and quercetin methyl ethers, together with a few 6-hydroxyflavone and -flavonol derivatives. In addtion, a phloroacetophenone was identified. Similar substitution trends have earlier been observed for *A. olivierana* J. Gay ex. Bess of sect. *Seriphidium* (Wollenweber and Rustaiyan, 1991). *Artemisia aucher*i Boiss. of the same section differs in its flavonoid composition, but shares the formation of phloroacetophenones (Wollenweber *et al.*, 1992). Most species of sect. *Seriphidium* are extreme xerophyts (Bremer and Humphries, 1993). As could be shown in a comparable group of *Achillea* spp. (Valant-Vetschera and Wollenweber, 1994), species and group specifity of exudate flavonoid profiles may be quite low in extremely xerophytic taxa. Thus the encountered chemical differences in the few samples analysed are not at all surprising.

The diversity in terms of flavonoid substitution trends is quite high within the genus *Artemisia*. At least within sect. *Abrotanum*, the substitution trends might prove to be group specific. There is also quite a good congruence observed between botanical and other chemical characters. Once more it is proved that the formation of exudate flavonoid aglycones is related to the habitat, since most of the taxa analysed are either alpine species or prefer xeric habitats (see Wollenweber and Valant-Vetschera, 1995).

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