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FLAVONOID AGLYCONES FROM THE LEAF SURFACES OF SOME *ACHILLEA* SPECIES

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Key Word Index—*Achillea*; Compositae; Anthemideae; aerial parts; flavonoid aglycones; external accumulation; 6-methoxyflavonoids.

Abstract—Acetone washes of leaves and stems of *A. grandifolia*, *A. kotschyi*, *A. umbellata* and *A. spinulifolia* yielded methyl ethers of 6-hydroxyflavones and 6-hydroxyflavonols. Their structures were confirmed by spectroscopic methods and by co-chromatography with authentic standards. The ecological significance of their external accumulation is briefly discussed.

INTRODUCTION

The genus *Achillea* comprises more than 100 species which are mainly distributed in the northern hemisphere, with a preference for xeric or alpine habitats [1]. Previous studies on flavonoids of *Achillea* species have revealed that *C*-glycosylflavones, flavonol 3-*O*-glycosides and flavone 7-*O*-glycosides are of widespread occurrence within this genus [2]. In addition, free flavonoid aglycones were frequently detected in the leaf extracts. Only recently we noticed that these aglycones accumulate externally on leaves and stems, together with other lipophilic material [3]. As part of the systematic analysis of *Achillea* flavonoid profiles [4, 5], four species have now been examined for their aglycone composition.

RESULTS AND DISCUSSION

The species investigated belong to different taxonomic groups within *Achillea*: *A. grandifolia* Friv. and *A. kotschyi* Boiss. are members of section *Millefolium*, whereas *A. umbellata* (Sibth. and Smith) Boiss. is grouped within section *Ptarmica* and *A. spinulifolia* Fenzl ex Boiss. within section *Santolinoideae*, respectively. Comparative TLC of the acetone washes of aerial parts (leaves and stems) revealed the presence of various 6-methoxyflavones and 6-methoxyflavonols. However, even the major flavonoids can hardly be identified directly in these solutions because of the large amounts of terpenoids and waxy materials which form more than 90% of the exudate. Bulk material of the four species was therefore rinsed with acetone and the concentrated washings were defatted by methanol treatment. Flavonoids were separated from terpenoids by passage over Sephadex LH-20 and the flavonoid fractions were then analysed as usual (see Experimental).

The acetone wash of a Turkish collection of *A. grandifolia* contained 6-hydroxyluteolin 6-methyl ether (nepetin), the coumarin esculetin, and a flavonol that was assumed to be a quercetin dimethyl ether ($M^+ = 346$; $M - 15 < M^+$). Its chromatographic properties and UV spectra indicated the presence of two methyl groups in positions 3 and 6. The assumed structure was confirmed by direct comparison with an authentic sample of quercetagetin 3,6-dimethyl ether (axillarin). The same compound was present in a population of *A. kotschyi*‡ collected in Bulgaria. The aglycone profile of this material consisted of scutellarein 6-methyl ether (hispidulin), 6-hydroxyluteolin 6-methyl ether (nepetin), 6-hydroxykaempferol 3,6-dimethyl ether, quercetagetin 3,6,3′-trimethyl ether (jaceidin) and quercetagetin 3,6,7,4′-tetramethyl ether (casticin). Traces of luteolin, kaempferol 3-methyl ether (isokaempferide), kaempferol 3,7-dimethyl ether (kumatakenin) and quercetin 3-methyl ether were also detected by TLC. *Achillea umbellata* (cultivated material) yielded nepetin, 6-hydroxykaempferol 3,6-dimethyl ether, 6-hydroxykaempferol 3,6,4′-trimethyl ether and quercetagetin 3,6,4′-trimethyl ether (centaureidin). Trace amounts of apigenin, luteolin, kaempferol 3,4′-dimethyl ether (ermanin) and axillarin were also present. The aglycone profile of *A. spinulifolia* (cultivated material) was dominated by flavones with a few flavonols in addition. Major flavonoids present were scutellarein 6,7-dimethyl ether (cirsimaritin), salvigenin, eupatorin, 6-hydroxyluteolin 6,7,3′,4′-tetramethyl ether, casticin and quercetagetin 3,6,7,3′,4′-pentamethyl ether (artemetin). Hispidulin, scutellarein 6,4′-dimethyl ether (pectolinarigenin), nepetin and 6-hydroxykaempferol 3,6,7,4′-tetramethyl ether were detected as minor constituents.

The flavonoid profiles observed so far within *Achillea* [3] largely resemble those known from other genera of the Compositae in the predominance of 6-methoxyflavonoids [7]. Furthermore their external accumulation has been frequently reported for this family [7] and this phenomenon is of increasing interest. In many cases a correlation between preferred habitat (xeric, alpine) and the production of excreted flavonoids has been noted, indicating

‡According to a taxonomic revision of the *A. nobilis* group [6] the name *A. urumoffii* Hal. is synonymous with *A. kotschyi*, which is the older and therefore the valid taxonomic name.

that these compounds may be of ecological significance [8–10]. As similar correlations are also apparent within *Achillea*, the present results are in agreement with this line of reasoning.

Three lipophilic 6-methoxyflavonoids were reported recently from aerial parts of *A. santolinoidea* [11]. According to the results of our studies on this genus [3, 12] it is certain that they are also constituents of a leaf exudate. The use of aglycone profiles as additional chemical features in *Achillea* is suggestive, but it is highly dependent on a detailed study of their species specificity, a project which is currently under way.

EXPERIMENTAL

Achillea grandifolia was collected in North Turkey (Keltepe, leg. H. Aksoy, 1985; voucher at Institute of Pharmacognosy, Vienna) and *A. kotschyi* in Bulgaria (Stara planina; leg. S. Ivancheva and B. Kuzmanov, Nov. 1985; voucher at Botany Institute, Bulgarian Academy of Science, Sofia, sub *A. urumoffii*). *A. umbellata* was cultivated (Department of Systematic Botany, University of Lund, Sweden; voucher in Lund) and *A. spinulifolia* also (Botanical Garden, University of Vienna; voucher in Vienna, WU).

Dried aerial parts (leaves and stems) were rinsed with Me_2CO to dissolve the epicuticular material. The concentrated solutions were dissolved in boiling MeOH. Fatty and waxy material deposited on cooling and was eliminated by centrifugation. The remainder was dissolved in MeOH and passed over columns with Sephadex LH-20, eluted with MeOH, to separate the flavonoids from the major amounts of terpenoids which were not analysed further. The total phenolic portion was so minute that the flavonoids were mostly identified by direct comparisons with authentic markers on silica gel and on polyamide [13]. Individual compounds could only in a few cases be isolated by preparative TLC on silica. Their UV and MS data confirmed these identifications [14].

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REFERENCES

1. Heimerl, A. (1884) *Denkschr. Kaiserl. Akad. Wiss., Math.-Naturwiss. Kl.* **48**, 113.
2. Valant, K. (1978) *Naturwissenschaften* **65**, 437.
3. Valant-Vetschera, K. M. and Wollenweber, E. (1986) *Proc. 7th Int. Biofl. Symp.*, Szeged, Hungary, May 1985.
4. Valant-Vetschera, K. (1985) *Biochem. Syst. Ecol.* **13**, 119.
5. Valant-Vetschera, K. (1987) *Biochem. Syst. Ecol.*, in press.
6. Bässler, M. (1963) *Feddes Repert.* **68**, 139.
7. Wollenweber, E. and Dietz, V. H. (1981) *Phytochemistry* **20**, 869.
8. Wollenweber, E. (1984) *Z. Naturforsch.* **39c**, 833.
9. Wollenweber, E. (1985) *Pl. Syst. Evol.* **150**, 83.
10. Proksch, P. and Rodriguez, E. (1985) *Biologie in unserer Zeit* **15**, 75.
11. Sanz, J., Martinez-Castro, I. and Pinar, M. (1985) *J. Nat. Prod.* **48**, 993.
12. Valant-Vetschera, K. M. and Wollenweber, E., *Biochem. Syst. Ecol.*, submitted.
13. Wollenweber, E. (1982) *Supplement Chromatography* 1982. GIT, Darmstadt.
14. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.