Phytochemistry, Vol. 27, No. 12, pp. 3849–3851, 1988.
Printed in Great Britain.

0031–9422/88 $3.00+0.00

FLAVONOID DISTRIBUTION IN *ARNICA* SUBGENERA *MONTANA* AND *AUSTROMONTANA*

MARION EBERT, IRMGARD MERFORT and GÜNTER WILLUHN

Institut für Pharmazeutische Biologie der Universität Düsseldorf, 4000 Düsseldorf, F.R.G.

(*Received in revised form* 8 *April* 1988)

Key Word Index—*Arnica*; subgenera *Montana* and *Austromontana*; Asteraceae; flavonoids; chemotaxonomy.

Abstract—Twenty flavonoid aglycones have been identified from *Arnica acaulis*, subgenus *Montana* and *A. nevadensis* and *A. viscosa*, subgenus *Austromontana*. The chemotaxonomic significance of the results is discussed.

INTRODUCTION

The genus *Arnica* L. consisting of *ca* 32 species is divided by Maguire into five subgenera: *Arctica*, *Austromontana*, *Chamissonis*, *Montana*, and *Andropurpurea*. As part of a chemotaxonomic study of the genus *Arnica* we reported previously on the flavonoid aglycones found in five of the seven species of the subgenus *Chamissonis* and of *A. montana*, subgenus *Montana* [1–4]. Continuing this study we have now identified the flavonoid aglycones in *A. acaulis* (subgenus *Montana*), *A. nevadensis* and *A. viscosa* (both subgenus *Austromontana*).

RESULTS AND DISCUSSION

Nine flavonoid aglycones were isolated from *A. acaulis*, subgenus *Montana* and 18 from subgenus *Austromontana*. They were identified as flavones, flavonols, flavanones and their methyl ethers, some of them with methylation at C-6, C-7 or C-6 and 7. The methods used for identification were UV spectral analysis, MS, ^{1}H NMR and co-chromatography with standard compounds, when available.

Subgenus *Montana* is represented by only two species: *A. montana* from Europe and *A. acaulis* which is restricted to southeast North America. According to Maguire the latter is thought to be a "highly anomalous population having no near relatives in North America" [5]. It might be an offshoot of *A. montana* but the flavonoid aglycones found in the flowers of the two species are very different. Thus, the flavonoid profile of *A. montana* consists of seven flavones and nine flavonols, ten of which are 6-methoxylated (Table 1). While in the flowers of *A. acaulis* (Walt.) only nine aglycones were identified: one flavonol, 6-methoxykaempferol (**10**) and eight flavones (**6**, **8**, **18–22**, **24**). The replacement of flavonols by flavones is considered as an advanced feature [6]. Methoxylation at C-6 (**6**, **8**, **10**) or methylation at C-7 (**18**, **19**) or that at both positions (**20–22**) was observed.

Subgenus *Austromontana* is divided by Maguire into two sections: *Eulatifoliae* and *Eradiatae* [5]. This division is supported by Straley [7], who, in addition, creates a further subgenus *Calarnica* including *A. venosa* and *A. viscosa*. In a recent study, Wolf and Denford reject Maguire's sections and Straley's new subgenus as artificial, because they recognize no infraspecific taxa particularly with respect to flavonoid chemistry [8].

Arnica nevadensis A. Gray (sect. *Eulatifoliae*) is a high mountain species of the Sierra Nevada of California extending northward to the north Cascades and Olympic Mountains of Washington [5]. According to [5, 9] this species is probably derived from *A. cordifolia* and from species of another subgenus. Its leaf flavonoid profile (Table 1) consists of flavonols and flavones mainly with 6-methoxylation. It is quite similar to that found in species of the subgenus *Chamissonis* [1] supporting the idea that *A. nevadensis* might be derived from species of this subgenus. However, further studies are needed to confirm this proposal.

Arnica viscosa A. Gray (sect. *Eradiatae*) is one of the rarest and most distinct species of the genus *Arnica*. Only seven populations on high alpine volcanic slopes are known. According to Wolf and Denford [9] it is probably the most recently derived species of *Austromontana*. Flowers as well as the aerial parts of *A. viscosa* were examined but organ specific variation was not observed. The flavonoid profile differed from that of *A. nevadensis* in the predominance of flavones, supporting its advanced state, and flavanones, both with 7-methylation (**16–19**, **26–28**). It is interesting that in all species regarded as advanced in the subgenera [5] such as *A. acaulis*, *A. viscosa* and *A. mollis* several flavones with 7-methylation were found.

In a previous flavonoid study of the subgenus *Austromontana* Wolf and Denford [9] examined all the above mentioned species. However, their results did not agree with ours in that we could not detect the same flavonoids in all cases and the number of compounds they found was much smaller. Perhaps flavonoid variation in different populations found by them [9] in species of *Austromontana* is responsible for this discrepancy, although the two populations from *A. nevadensis* and *A. viscosa* they studied showed no variation. According to ref. [8], all known collections of *A. viscosa* showed virtually no morphological interpopulational variation. Further studies must be undertaken to show if this is also the case for flavonoid constituents. In our opinion it is not possible to decide at present on the correct division of subgenus *Austromontana* although the flavonoid profiles of both

Table 1. Distribution of flavonoid aglycones in *Arnica*

Species	(1) Ap	(2) Km	(3) Qu	(4) Chrysoeriol (Lu 3'-Me)	(5) Isorhamnetin (Qu 3'-Me)	(6) Hispidulin (Ap 6-OMe)	(7) Pectolinarigenin (Ap 4'-Me, 6-OMe)	(8) Eupafolin (Lu 6-OMe)	(9) Jaceosidin (Lu 3'-Me, 6-OMe)	(10) Km 6-OMe	(11) Betuletol (Km 4'-Me, 6-OMe)	(12) Patuletin (Qu 6-OMe)	(13) Spinacetin (Qu 3'-Me, 6-OMe)	(14) Laciniatin (Qu 4'-Me, 6-OMe)
Montana	●	●	●	●	●	●	●	●	●	●	●	●	●	●
*A. montana**														
*A. acaulis**						●		●		●				
Austromontana														
Sect. *Eulatifoliae*														
A. nevadensis†		●	●			●	●	●	●	●			●	
Sect. *Eradiatae*														
*A. viscosa**†		●	●											

Key: Ap, apigenin; Lu, luteolin; Km, Kaempferol; Qu, quercetin; Nar, naringenin; Eri, eriodictyol; *in flowers, †in leaves.

species examined is quite different. Additionally, a previous study of sesquiterpene lactones showed that only eudesmanolides occur in *A. nevadensis* and pseudoguaianolides in *A. viscosa*; constituents which are formed by different biosynthetic routes.

EXPERIMENTAL

Plant material. A. acaulis (Walt.) was collected in 1985 in North Carolina. A voucher specimen is deposited at the herbarium of the University of North Carolina at Chapel Hill, U.S.A. *A. nevadensis* A. Gray was harvested in Lassen Volcanic National Park, California, U.S.A. and *A. viscosa* A. Gray near Mt. Shasta, California, U.S.A., in 1979. Voucher specimens are deposited at the herbarium of the Institute of Pharmaceutical Biology, University of Düsseldorf.

Fractionation and identification. Air-dried powdered flowers of *A. acaulis* (40 g) were extracted with CH_2Cl_2. The extract (3.8 g) was chromatographed on Sephadex LH-20 (Pharmacia) with MeOH. Subsequent sepn on silica gel TLC plates with $CHCl_3$–MeOH (15:1) or *n*-pentane–Et_2O (8:17) afforded **6** (6 mg), **8** (1 mg), **10** (1 mg), **18** (2 mg), **19** (2 mg), **20** (2 mg), **21** (> 1 mg), **22** (1 mg), **24** (> 1 mg).

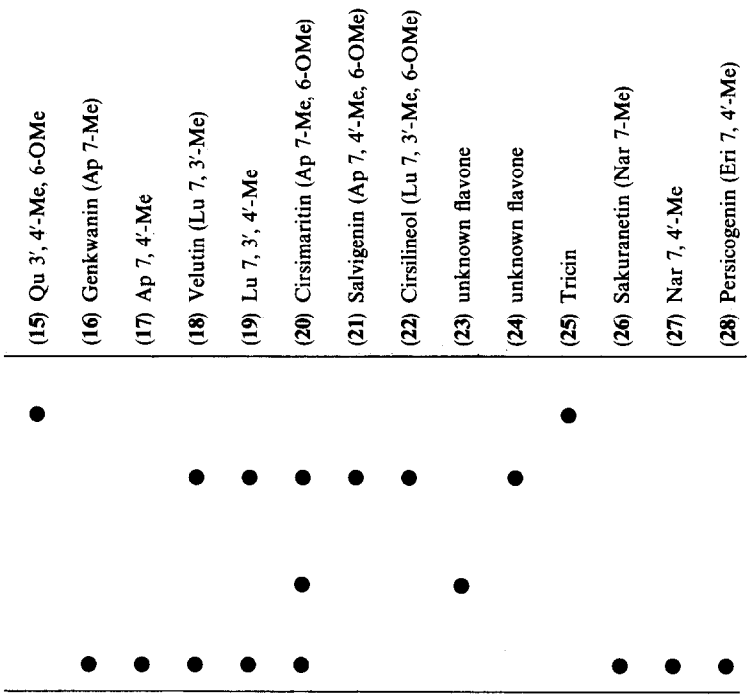
Air-dried powdered above ground parts of *A. viscosa* (1070 g) were extracted with $CHCl_3$. After removing the solvent, the residue was dissolved in aq. EtOH (60%). The aq. ethanolic extract was concd under red. pres. until only H_2O remained which was re-extracted with $CHCl_3$ (122 g) and chromatographed on Sephadex LH-20 with MeOH. Fractions with flavonoids were further separated over silica gel columns using $CHCl_3$–MeOH with increasing amounts of MeOH. Subsequent separation on self-made TLC plates (silica gel 60 PF_{254}, 0.5 mm) with $CHCl_3$–MeOH (15:1) and crystallization afforded **16** (4.2 mg), **17** (31.5 mg), **18** (25.3 mg), **19** (9.1 mg), **20** (1.9 mg), **26** (5.7 mg), **27** (8.4 mg), **28** (11.8 mg).

Flowers of *A. viscosa* were extracted with petrol–Et_2O (1:2), then with MeOH. MeOH was added to the petrol–Et_2O extract at −17° to remove long chain compounds (16.38 g extract) [10]. After addition of H_2O to the MeOH extract organic solvent was removed under red. pres. and the remaining H_2O extracted with CH_2Cl_2 (3.5 g extract). In addition to **2** (2 mg) and **3** (5 mg), **16**–**20** and **26**–**28** were isolated from these two extracts as described above.

Leaf extracts of *A. nevadensis* (296 g) were prepared as for flower extracts of *A. viscosa* and the compounds isolated as described for *A. acaulis*. The following flavonoids were yielded: **6** (7.7 mg), **7** (4.5 mg), **9** (4 mg), **10** (8.6 mg), **13** (1.5 mg), **20** (3.7 mg), **23** together with **7** (2.4 mg). Compounds **2**, **3** and **8** were identified by TLC. Finally all isolated compounds were purified on columns of Sephadex LH-20 with MeOH as eluate to remove silica gel residues.

Acknowledgements—This study was supported by the Deutsche Forschungsgesellschaft. We are grateful to Dr Massey (University of North Carolina, Chapel Hill) for collecting flowers of *A. acaulis*, Professor Wollenweber (Universität Darmstadt) for an authentic sample of persicogenin, Professor Brieskorn, Universität Würzburg for cirsimaritin, PD. Mues, Universität des Saarlandes for cirsilineol, Dr. Wendisch, Zentralbereich Forschung und Entwicklung der Bayer AG, Leverkusen for taking the

subgenera *Montana* and *Austromontana*



360 MHz ^{1}H NMR spectra, Professor Bornowski and Dr. Reimann, Institut für Organische Chemie, TU Berlin for the 400 MHz ^{1}H NMR spectra and Mr. Bürgel, Institut für Anorganische Chemie, Universität Düsseldorf for the mass spectra.

REFERENCES

1. Merfort, I., Marcinek, C., Eggert, A. (1986) *Phytochemistry* **25**, 2901.
2. Merfort, I. (1984) *Planta Med.* 107.
3. Merfort, I. (1985) *Planta Med.* 136.
4. Merfort, I. and Wendisch, D. (1988) *Planta Med.* **54**, 247.
5. Maguire, B. (1943) *Brittonia* **4**, 386.
6. Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*. Academic Press, London.
7. Straley, G. B. (1980) Ph.D. Diss., Univ. Brit. Columbia.
8. Wolf, S. J. and Denford, K. E. (1984) *Rhodora* **86**, 239.
9. Wolf, S. J. and Denford, K. E. (1984) *Biochem. Syst. Ecol.* **12**, 183.
10. Jakupovic, J. (1979) Dissertation. TU Berlin.
11. Ebert, M. and Willuhn, G. (1987) *Pharm. Weekbl.* **9**, 232.