Rare Flavonoid Aglycones from *Anaphalis margaritacea* and Two *Gnaphalium* Species

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*Anaphalis margaritacea*, *Gnaphalium chilense* and *G. microcephalum* have been analyzed for flavonoid aglycones deposited externally on aerial parts. *Anaphalis* was found to produce, among others, several rare flavonoids, all of which are O-substituted in position 6 and mostly also in position 8. Both *Gnaphalium* species exhibit 3,5-dihydroxy-6,7,8-trimethoxyflavone as the major exudate flavonoid.

**Introduction**

*Anaphalis margaritacea* (L.) B. and H. (syn. *Gnaphalium margaritaceum* L.), the Pearly Everlasting, is a herbaceous plant native to N. America. The herb is used as an expectorant and astringent, especially in homeopathy. Several *Gnaphalium* species, *G. keriense*, *G. polyccephalum* and *G. uliginosum*, are also used therapeutically [1], but no such use is known for the two species analyzed here, *Gnaphalium chilense* Sprengel and *G. microcephalum* Nutt. As members of the Asteraceae family, tribe Inuleae, with more or less aromatic scent, these plants were assumed to exhibit exudate flavonoids on their leaves and stems, although these are more or less coated with dead hairs. Analysis of plant material cultivated in a Botanical Garden and collected in the field in California, respectively, confirmed this assumption. In the following we report the flavonoid aglycones found in this material.

**Materials and Methods**

*Anaphalis margaritacea* was cultivated in the Botanical Garden at Darmstadt. Leafy stems and inflorescences were collected separately. Airdried material was briefly rinsed with acetone to dissolve the exudate. When TLC revealed that both portions exhibited the same flavonoid pattern, they were combined. A total of 580 g dry aerial parts yielded 7.1 g of a brownish resin, covered with a layer of waxy material. *Gnaphalium chilense* was collected in Humbold County, CA, in Clam Beach County Park in fixed dunes back from the water. A voucher (G. and K. Yatskievych 88–178) is kept at the Missouri Botanical Garden herbarium (MO). *G. microcephalum* was collected in Mendocino County, CA. A voucher (G. and K. Yatskievych 88–165) is kept at MO. Again the exudate material was obtained by rinsing air-dried material with acetone. Fatty and waxy components were eliminated in all cases by dissolving the exudate in boiling methanol, allowing the solution to cool and centrifuging. The residue was then passed over Sephadex LH-20, eluted with methanol, to separate the flavonoids from the predominant terpenoids.
Fractions were monitored by TLC on silica with solvents A (toluene–methyl ethyl ketone 9:1) and B (toluene–methyl ethyl ketone–glacial acetic acid 18:5:1) and on polyamide DC-11 with solvents C (petrol100–140°C–toluene–methyl ethyl ketone–methanol 12:6:2:1) D (toluene–petrol100–140°C–methyl ethyl ketone–methanol 12:6:2:1) and E (toluene–methyl ethyl ketone–methanol 12:5:3). Chromatograms were viewed in UV366 before and after spraying with “Naturstoffreagenz A” (NA; for flavonoids) and with MnCl2 reagent [2] (for terpenoids; used on silica only). Flavonoid-containing fractions of each plant species were combined as far as possible and studied further after column chromatography on polyamide and on acetylated polyamide. Several flavonoids crystallized from the corresponding fractions, and a further one was isolated by preparative TLC on silica in solvent B. One major flavonoid crystallized directly from non-polar fractions of *Gnaphalium chilense*.

Electron impact mass spectra (EIMS) were recorded at 70 eV. 1H and 13C NMR spectra were recorded in CDCl3 at 200 MHz and 50 MHz, respectively. Melting points are uncorrected.

**Results**

From the leaf and stem exudate of *Anaphalis margaritacea* a number of flavonoid aglycones was isolated. Galangin-3-methyl ether, 6-hydroxygalangin-3,6-dimethyl ether (compound 1), 8-hydroxygalangin-3,8-dimethyl ether (gnaphalin; compound 2), and quercetin were obtained in crystalline form. Compounds 3–6 were also crystalline, though some in very small amount only. Compound 7 was isolated by preparative TLC on silica. The structures of products 1 and 2 were corroborated and products 3–7 were analyzed by UV, MS and NMR. In addition galangin and 8-methoxy galangin were identified by direct comparisons with authentic samples.

Compound 1 formed fine yellow crystals, m.p. 172°C. It appeared as a dark spot (UV366) on polyamide at Rf 0.57 (solvent D) that turned dark brown with NA. MS m/z (rel. int.) 314 (54, M+; C17H14O6), 299 (100), 271 (30), 256 (25), 139 (30), 118 (19), 105 (33). The intensity ratio of the [M–15]+ and M+ peaks indicated 6-methoxy substitution in compound 1 and 8-OMe substitution in compound 2. Direct comparisons with markers reveal that they were identical to 6-hydroxygalangin-3,6-dimethyl ether (1) and to 8-hydroxygalangin-3,8-dimethyl ether (2), respectively. Their identities were further corroborated by their 1H NMR spectra (see Table I). Compound 3 formed light yellow crystals, m.p. 254–258°C. It formed a reddish-brown TLC spot at Rf 0.38 (polyamide, solvent D) that turned greenish after spraying with NA. MS m/z (rel. int.) 300 (100, M+; C16H12O6), 285 (26), 282 (39), 257 (87), 105 (92). This spectrum suggested a flavonol with three hydroxy groups and one methoxy group. The 1H NMR spectrum also showed signals for a methoxy group and three hydroxy groups exchangeable with D2O and indicated an unsubstituted phenyl ring. A NOE effect between 5-OH and a methoxy group ensured the C-6 position of...
the latter. The structure of compound 3 was thus deduced to be 3,5,7-trihydroxy-6-methoxy flavone (alnusin). Its identity was confirmed by direct comparison with an authentic sample [3].

Compound 4 formed fine yellow crystals, m.p. 190 °C and produced a dark spot that turned reddish-brown (polyamide TLC, solvent D) with Na at Rf 0.44. MS m/z (rel. int.) 330 (79, M⁺; C₁₇H₁₄O₇), 315 (100), 287 (36), 272 (21), 105 (40), 77 (57), 69 (25). The mass spectrum indicated this flavonoid to be a flavone/flavonol with three hydroxy and two methoxy groups, for which the intensity of [M -15]+ suggested 8-OMe-substitution in accord with two methoxy and the hydroxy signals which appeared in the ¹H NMR spectrum. The relative position of these groups followed from the NOE effects between 6-OMe and 5-OH and between 8-OMe and H-2'/6', respectively. Compound 4 is thus shown to be 3,5,7-trihydroxy-6,8-dimethoxy flavone.

Compound 5 formed orange-yellow crystals, m.p. 177 °C. It appeared as a dark spot at Rf 0.67 (polyamide TLC, solvent D) before and after spraying with Na. MS m/z (rel. int.) 344 (67, M⁺; C₁₈H₁₆O₄), 329 (100), 301 (13), 286 (8), 105 (36), 89 (18), 77 (20), 69 (21). Again MS fragmentation indicated an 8-methoxy flavone or flavonol with prenyl side chain: 5.26, br t (J = 7 Hz); 3.36, br d (J = 7 Hz, 2H); 1.82 and 1.76, br s (3H). The ¹H NMR spectrum thus revealed the presence of a flavone with a phenyl group. An NOE effect was observed between 5-OMe and the prenyl group. Compound 6 is thus 5,7-dihydroxy-6-prenyl flavone.

Compound 7 was isolated in very small amount only, was not crystalline and formed a dark spot at Rf 0.20 (polyamide TLC, solvent D) that turned pale brown with Na. MS m/z (rel. int.) 324 (100, M⁺; C₁₈H₁₆O₄), 281 (39), 269 (63), 219 (18), 205 (66), 192 (19), 176 (30), 165 (100). Its molecular mass did not correspond to any flavonoid aglycone bearing the normal OH- and OMe-substituents and its chromatographic behaviour indicated a flavanone or dihydrochalcone structure. ¹H NMR δ ppm: 12.40 (5-OH); 7.47–7.35, m (H-3’,4’,5’); 6.14, br s (7-OH); 6.02, s (8-H); 5.40, dd (J = 3 and 13 Hz; 2-H); 3.08, dd (J = 13 and 17 Hz) and 2.83, dd (J = 3 and 17 Hz) (3-H); prenyl side chain: 5.26, br t (J = 7 Hz); 3.36, br d (J = 7 Hz, 2H); 1.82 and 1.76, br s (3H). The ¹H NMR spectrum thus revealed the presence of a flavanone with a phenyl group. An NOE effect was observed between 5-OMe and the prenyl group. Compound 6 is thus 5,7-dihydroxy-6-prenyl flavanone.
rated “waxy” material. Compound 8 formed deep yellow needles or platelets, m.p. 145–151°C. On polyamide TLC in solvent D it appeared as a yellow spot at Rf 0.88 that turned reddish-brown on spraying with NA. In daylight the spot was dark ochre/salmon-pink. MS m/z (rel. int.) 344 (100, [M]+; C_{18}H_{14}O_{9}), 329 (83; [M]+), 301 (24; [M–MeCOO]+), 105 (29). According to its molecular mass it is a flavone or flavonol with two hydroxy and three methoxy groups. The ^1H NMR spectrum showed three OMe signals, two OH signals (exchangeable with D_2O), and two aromatic CH signals: a two proton signal showing ortho and meta coupling (H2, H6) and a three proton multiplet (H3, H4, H5), all of which suggested a substituted galangin structure for 8. ^13C NMR δ ppm 145.6* (C-2), 136.4* (C-3), 176.0 (C-4), 147.8 (C-5), 136.0* (C-6), 153.3 (C-7), 133.2 (C-8), 145.2* (C-9), 105.4 (C-10), 130.8* (C-1’), 127.7 (C-2’/6’), 128.7 (C-3’/5’), 130.5* (C-4’); 61.2, 61.8, 62.1 (6,7,8-OMe) (*, †*: signals may be interchanged). The ^13C NMR spectrum confirmed an unsubstituted B-ring and a tri-OMe A-ring in good agreement with the spectra of several compounds having similarly substituted A rings [4] of B rings [5]. Compound 8 was thus shown to be 3,5-dihydroxy-6,7,8-trimethoxy flavone, its identity further confirmed by direct comparisons with an authentic sample [6]. Apart from this major flavonoid, the exudate of G. chilense exhibits only trace amounts of three unidentified flavonoids.

The exudate of Gnaphalium microcephalum contains only a very small flavonoid portion. Again 3,5-dihydroxy-6,7,8-trimethoxy flavone is the major flavone. In addition, 8-hydroxygalangin-3,8-dimethyl ether (gnaphalin, compound 2), galangin, 6-hydroxykaempferol-6′,4′-dimethyl ether and a trace amount of 6-methoxy galangin (alnustinol) were identified by direct comparisons with authentic samples. Several further flavonoid constituents remain unidentified.

**Discussion**

Apart from a few trivial flavonoids, most of the exudate constituents reported herein are more or less rare products. To our knowledge, 6-hydroxygalangin-3,6-dimethyl ether (1) has been found in nature only once before, in *Helichrysum heterolasiadum* [7]. The isomeric 8-hydroxygalangin-3,8-dimethyl ether (gnaphalin, 2) has so far been found in three Achyrocline species [8–10] and in *Gnaphalium robustum* [11]. 3,5,7-Trihydroxy-6-methoxy flavone or 6-methoxy-galangin (3) was first isolated from male flowers of *Alnus sieboldiana* and named alnustinol [12]. Later it was reported from three Asteraceae, namely *Chromolaena chaslaea* [13], *Baccharis bigelovii* [14], and *Cassinia quinquefaria* [5]. 3,5,7-Trihydroxy-6,8-dimethoxy flavone (4) has been found in nature only once before, in *Gnaphalium obtusifolium* [15]. 5,7-Dihydroxy-3,6,8-trimethoxy flavone (araneol, 5) was reported previously from *Anaphalis araneosa* [16], from *Gnaphalium elegans* [17], and from *Helichrysum decumbens* [18]. 5,7-dihydroxy-6-prenyl-flavone (6) was reported previously from *Helichrysum thapsus* [19] and from *H. rugulosum* [20]. 2′,4′,6′-Trihydroxy-3′-prenyl-chalcone (7) was first found in Pleiotaxis rugosa [21], then in *Helichrysum atrixiifolium* [22] and in *H. rugulosum* [20]. 3,5-Dihydroxy-6,7,8-trimethoxy flavone (8) was reported previously from *Helichrysum aran­rium* [23], *Achyrocline bogotensis* [24], *Achillea nobilis* [25], *Helichrysum decumbens* [18].

It is evident that except for *Pleiotaxis* in the Mutisieae (compound 7) and apart from two sources for compound 8, all plant sources cited herein belong to the tribe Inuleae and hence members of the Inuleae have a tendency to synthesize and accumulate these flavonoids. Our results confirm the earlier observation that in Inuleae flavonols with 6- and/or 8-O-substitution and lacking B-ring substitution are predominant. It must be kept in mind however that *Gnaphalium* and *Helichrysum* (sub­tribe Gnaphaliinae) remain the two best studied genera.

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