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FLAVONOIDS AND TRITERPENOIDS FROM *Anaphalis margaritacea*

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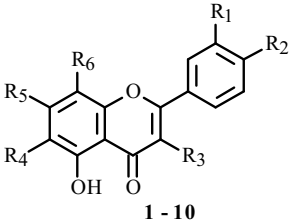
We have carried out systematical phytochemical studies on several medicinal herbs used in traditional Tibetan medicine growing only at 2500–4000 m above sea level on Qinghai-Tibet Plateau and found that all these plants are abundant in flavonoids [1–5]. The genus *Anaphalis* (Compositae) is widely distributed in the west and southwest regions of China, and 17 species of them are used as medicinal plants. *Anaphalis margaritacea* (Linn.) Benth. et Hook. f. has long been used by local inhabitants as a Tibetan medicine for cough and respiratory problems as well as for colds and rheumatism. Previous chemical investigation of this plant only revealed the presence of flavonoids, polyacetylenes, and hydroxylactone [6–9].

Herein we report the results of a thorough phytochemical study on flavonoids and triperpenoids from the whole plant of *Anaphalis margaritacea* (Linn.) Benth. et Hook. f. and their free-radical-scavenging potentials. Isolation of various fractions of the ethanol extract by column chromatography gave 12 known flavonoids. They were apigenin (**1**) [10], quercetin (**2**) [2, 8], kaempferol-3-*O*-β-D-glucopyranoside (**3**) [9], 3-methylquercetin (**4**) [5], spiraein (**5**) [10], 5,7-dihydroxy-3-methoxyflavone (**6**) [11], 5,7-dihydroxy-3,6,8-trimethoxyflavone (**7**), 5,7-dihydroxy-3,8-dimethoxyflavone (**8**), 5,6-dihydroxy-3,7-dimethoxyflavone (**9**) [4], 3,5-dihydroxy-6,7,8-trimethoxyflavone (**10**), tiliroside (**11**) [5] and kaempferol-3-*O*-[6″-*O*-(*trans*-*p*-coumaroyl)-4″-*O*-acetyl]-β-D-glucopyranoside (**12**) [12], together with four known triterpenoids, ursolic acid (**13**) [13], 3β-*O*-acetylursolic acid (**14**) [14], polnolic acid (**15**) [15], and 19α-hydroxy-3-acetylursolic acid (**16**) [16]. Their structures were established by direct comparison of their spectroscopic data with the corresponding literature values. Compounds **1**–**3**, **5**, **6**, **12**–**16** were isolated from *A*. *margaritacea* for the first time.



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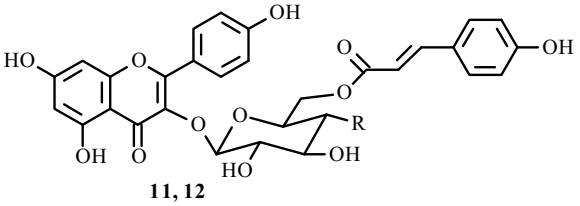

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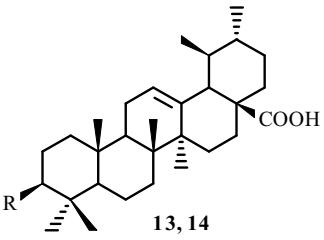

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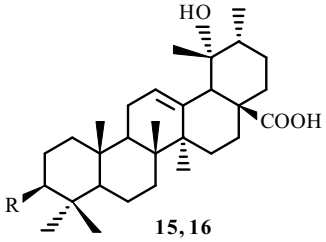

15, 16

1: $R_1 = R_3 = R_4 = R_6 = H$, $R_2 = R_5 = OH$; **2:** $R_1 = R_2 = R_3 = R_5 = OH$, $R_4 = R_6 = H$
3: $R_1 = R_4 = R_6 = H$, $R_2 = R_5 = OH$, $R_3 = OGlc$; **4:** $R_1 = R_2 = R_5 = OH$, $R_3 = OMe$, $R_4 = R_6 = H$
5: $R_1 = OGlc$, $R_2 = R_3 = R_5 = OH$, $R_4 = R_6 = H$; **6:** $R_1 = R_2 = R_4 = R_6 = H$, $R_3 = OMe$, $R_5 = OH$
7: $R_1 = R_2 = H$, $R_3 = R_4 = R_6 = OMe$, $R_5 = OH$; **8:** $R_1 = R_2 = R_4 = H$, $R_3 = R_6 = OMe$, $R_5 = OH$
9: $R_1 = R_2 = R_6 = H$, $R_3 = R_4 = OMe$, $R_5 = OH$; **10:** $R_1 = R_2 = H$, $R_3 = OH$, $R_4 = R_5 = R_6 = OMe$
11, 13, 15: R = OH, **12, 14, 16:** R = OAc

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TABLE 1. Free Radical Scavenging Activity of Isolated Compounds and VE in the DPPH Assay

Compound	IC_{20} (μM)	Compound	IC_{20} (μM)
1	>100	**7**	>100
2	6.36±0.11	**8**	>100
3	>100	**9**	>100
4	7.52±0.10	**10**	40.90±0.23
5	11.83±0.11	**11**	>100
6	>100	**12**	>100
VE	7.60		

Results are given as mean ±SD. Statistical analyses were performed using Student′s *t*-test.

For the evaluation of free-radical-scavenging properties of compounds **1**–**12**, a DPPH assay was adopted with vitamin E (VE) as positive control. In the DPPH system, the free radical scavenging activity of tested samples was expressed as IC_{20}. The results of scavenging activities of compounds **1**–**12** and VE are listed in Table 1; **2** (IC_{20}= 6.36±0.11 μM) and **4** (IC_{20}= 7.52±0.10 μM) was most active in the tested compounds. The higher scavenging activities were associated with the *ortho*-dihydroxyl B-ring structures in the flavonoids, conferring higher radical stability and participating in electron delocalization, as reported for other systems [2, 17].

General. The ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury-300BB NMR (300 MHz) spectrometers with TMS as internal standard. Silica gel (200–300 mesh) used for column chromatography (CC) and silica gel GF_{254} (10–40 μm) used for TLC were supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. China. VE was purchased from Aldrich, USA and DPPH from Sigma, USA.

Plant Material. The whole plant of *Anaphalis margaritacea* (Linn.) Benth. et Hook. f. was collected in Huzhu County, Qinghai province, P. R. China, in August 2005. It was identified by Dr. Huan-Yang Qi. The voucher specimens (2005A002) were deposited in the Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. Powdered and dried whole plant of *A. margaritacea* (2.6 kg) was extracted with 95% EtOH at room temperature. The combined extract (183 g) was then suspended in H_2O (1.0 L) and extracted successively with petroleum ether, EtOAc, *n*-BuOH (3 × 1.0 L each), respectively. The solvents were distilled to produce the petroleum ether fraction (40 g), the EtOAc fraction (43 g), and the *n*-BuOH fraction (26 g).

The petroleum ether extract was chromatographed on a silica gel (200–300 mesh, 450 g, 8 × 140 cm) column using petroleum ether–EtOAc. Elution gave six compounds, namely **6** (17.6 mg), **8** (21.3 mg), **9** (14.8 mg), **10** (12.4 mg), **14** (13.7 mg), and **16** (15.2 mg). The ethyl acetate extract was chromatographed on a silica gel (200–300 mesh, 500 g, 8 × 140 cm) column using petroleum ether–acetone. Elution of the column gave 10 compounds, namely **1** (21.3 mg), **2** (17.1 mg), **3** (12.4 mg), **4** (16.8 mg), **5** (24.8 mg), **7** (18.7 mg), **11** (30.3 mg), **12** (31.5 mg), **13** (19.6 mg), and **15** (12.7 mg).

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