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Pentacyclic triterpenoids from *Aster ageratoides* var. *pilosus*

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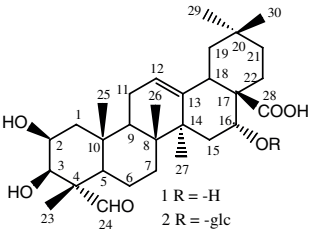
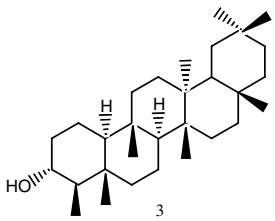
Two new pentacyclic triterpenoids, 2β,3β,16α-trihydroxyl-24α-al-olean-12-en-28-oic acid (**1**), 2β,3β-dihydroxyl-16-*O*-β-D-glucopyranose-24α-al-olean-12-en-28-oic acid (**2**) and two known pentacyclic triterpenoids were isolated from the roots of *Aster ageratoides* var. *pilosus.* Their structures were elucidated by spectroscopic methods (IR, MS, ^{1}H, ^{13}C and 2D NMR). In addition, the anti-bacterial activity and anti-tumor activity of compound **2** were tested.

1. Introduction

Aster ageratoides Turcz. (Compositae) is widespread in China. The whole plant has been used in a Chinese folk medicine for the treatment of cold, fever, tonsillitis, bronchitis, snake bite and bee sting. (Jiangsu New Medical College 1977). Two diterpene glycosides and their aglycone have been isolated from this plant previously (Cheng et al. 1993). However, phytochemical and pharmacological investigations of *A. ageratoides* var. *pilosus* have not been reported before. Here we report the isolation and structural elucidation of a new pentacyclic triterpene, 2β,3β,16α-trihydroxyl-24α-al-olean-12-en-28-oic acid (**1**) and a new pentacyclic triterpene glycoside, 2β,3β-dihydroxyl-16-*O*-β-D-glucopyranose-24α-al-olean-12-en-28-oic acid (**2**), together with two known pentacyclic triterpenoids (**3**, **4**).





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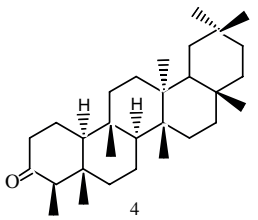

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The anti-bacterial activity of compound **2** was assayed against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli,* as was the anti-tumor activity of compound **2** against human leukemia (HL-60), human hepatoma (Bel-7402) and human ovarian neoplasm (HO-8910) cell lines were assayed.

2. Investigations, results and discussion

The EtOAc soluble part of the methanolic extract from the roots of *A. ageratoides* var. *pilosus* was rechromatographed over silica gel to afford four pentacyclic triterpenoids **1**–**4**. The known compounds were determined as friedelinol (**3**) (Ho et al. 1995) and friedelin (**4**) (Ho et al. 1995; Akihisa et al. 1992) by comparison of their spectral data (^{1}H ^{13}C NMR, DEPT and EIMS) with those reported in the literature respectively.

Compound **1** was obtained as a white powder, and its molecular formula was assigned as $C_{30}H_{46}O_6$ from HRESI MS $[M + NH_4]^+$ at m/z 520.3639 (calcd. 520.3633). The IR spectrum displayed peaks at 3445 (OH), 1720 (CHO), 1695 (COOH). Six singlet peaks (δ_H 0.84 s, 0.85 s, 0.96 s, 1.36 s, 1.52 s, 1.64 s, each 3 H) and one olefinic proton resonance as a broad singlet for H-12 at δ_H 5.47 in the ^{1}H NMR spectrum, as well as one carboxyl group signal (δ_C 180.0 s) and one trisubstituted double bond signals (δ_C 122.3 d, 145.2 s) in the ^{13}C NMR spectrum suggested that it should be a 12-en-28-oleanic acid type pentacyclic triterpenoid (Asada et al. 1989). In addition, its ^{1}H NMR spectrum exhibited an aldehyde signal at δ_H 9.42 (1 H, s) and three oxygenated methine signals at δ_H 3.90 (1 H, d, 2.7 Hz), 4.34 (1 H, m) and 5.05 (1 H, brs), which were supported by ^{13}C NMR and DEPT spectra. The fragmental ions of EI MS at m/z 238 and 264 resulting from retro-Diels-Alder cleavage of the C-ring indicated two hydroxyl groups and an aldehyde group at the A/B rings, and another hydroxyl group at the D/E rings. In the HMBC spectrum, the long-range correlations of H-1 (δ_H 2.20, m) with C-2 (δ_C 71.1, d) and C-3 (δ_C 72.2, d); H-3 (δ_H 3.90, d, 2.7 Hz) with C-4 (δ_C 55.6, s), C-23 (δ_C 11.0, q) and C-24 (δ_C 207.3, d); H-24 (δ_H 9.42, s) with C-4 and C-23; H-16

Table: ^{13}C NMR data of 1, 2, 3 and 4 (75 MHz, δ, ppm, TMS)[a]

Carbon	1	2	3	4
1	44.8 t	44.5 t	15.8 t	22.3 t
2	71.1 d	70.9 d	36.1 t	41.5 t
3	72.2 d	72.1 d	72.8 d	213.1 s
4	55.6 s	55.4 s	49.2 d	58.2 d
5	48.3 d	48.0 d	39.3 s	42.1 s
6	21.0 t	20.8 t	41.8 t	41.3 t
7	33.0 t	32.7 t	17.6 t	18.2 t
8	40.3 s	40.1 s	53.2 d	53.1 d
9	47.6 d	47.4 d	37.1 s	37.4 s
10	36.2 s	36.0 s	61.4 d	59.5 d
11	24.0 t	23.8 t	35.4 t	35.6 t
12	122.3 d	123.1 d	30.6 t	30.4 t
13	145.2 s	144.9 s	37.9s	38.3 s
14	42.3 s	41.7 s	38.4 s	39.6 s
15	35.8 t	32.3 t	32.4 t	32.4 t
16	74.4 d	79.3 d	35.6 t	36.0 t
17	48.9 s	48.2 s	30.0 s	30.5 s
18	41.5 d	41.2 d	42.9 d	42.8 d
19	47.3 t	46.3 t	35.2 t	35.3 t
20	31.0 s	30.7 s	28.2 s	28.1 s
21	36.3 t	36.0 t	32.8 t	32.8 t
22	33.0 t	32.3 t	39.7 t	39.2 t
23	11.0 q	10.8 q	11.6 q	6.8 q
24	207.3 d	207.3 d	16.4 q	14.6 q
25	16.9 q	16.7 q	18.2 q	17.9 q
26	17.5 q	17.3 q	18.6 q	18.6 q
27	27.3 q	27.2 q	20.1 q	20.1 q
28	180.0 s	179.4 s	32.1 q	32.1 q
29	33.3 q	33.2 q	31.8 q	31.8 q
30	24.7 q	24.6 q	35.0 q	35.0 q
1′		101.2 d		
2′		75.5 d		
3′		79.2 d		
4′		72.1 d		
5′		78.0 d		
6′		63.0 t		

[a] The spectra of **1** and **2** were measured in C_5D_5N, and those of **3** and **4** in $CDCl_3$

(δ_H 5.05, brs) with C-14 (δ_C 42.3, s) and C-18 (δ_C 41.5, d); H-16 and H-18 (δ_H 3.43, dd, 2.7, 10.5 Hz) with C-28 (δ_C 180.0, s); H-12 (δ_H 5.47, brs) with C-9 (δ_C 47.6, d) and C-18 were observed, showing that an aldehyde group was linked at C-4, a carboxyl group was linked at C-17, an olefinic bond was at C-12 and C-13, and three hydroxyl groups were attached at C-2, C-3 and C-16, respectively. The NOESY cross peak observed between H-3 (δ_H 3.90, d, 2.7 Hz) and H-5 (δ_H 1.47 m) implied that H-3 was axial, and 3-OH was equatorial with the β-configuration. Moreover, the small value of the coupling constant (J = 2.7 Hz) with the between H-3 and H-2 (δ_H 4.34, m), suggested that H-2 must be equatorial, while 2-OH was axial with the β-configuration. On the other hand, NOESY showed obvious throughspace interactions between H-23 (δ_H 1.52, s) and H-25 (δ_H 1.36, s), H-24 (δ_H 9.42, s) and H-3, which revealed that 24-CHO was equatorial with the α-configuration. The configuration of the 16-OH group of **1** was determined to be α because the ^{1}H NMR spectrum of **1** showed the signal of H-16 at δ 5.05 as a broad singlet (Sakai 1999). On the basis of the above evidence, the structure of compound **1** was determined to be 2β,3β,16α-trihydroxyl-24α-al-olean-12-en-28-oic acid.

Compound **2** was also obtained as a white powder, and showed the presence of carbonyl groups (1719, 1699), a hydroxyl group (3422) and a trisubstituted double bond (1640) in its IR spectrum. Its HRESI MS showed $[M + NH_4]^+$ at m/z 682.4163 (calcd. 682.4161), corresponding to a molecular formula $C_{36}H_{56}O_{11}$. The ^{13}C NMR and DEPT spectral data of **2** were very similar to those of compound **1**, except for one oxygenated methylene carbon signal at δ_C 63.0 and five oxygenated methine carbon signals at δ_C 72.1, 75.5, 78.0, 79.2 and 101.2. This suggested the presence of a sugar moiety (Inose et al. 1991). Acid hydrolysis of **2** with 2 M HCl afforded D-glucopyranose, which was identified by PC by comparison with an authentic sample. Furthermore, the ^{1}H NMR spectrum showed that the glucopyranosyl group was linked in the β-configuration for its anomeric proton at δ 4.82 (d, J = 7.5 Hz). Comparing the signals at C-16 of **2** with those in **1**, **a** glycosylation shift of +4.9 ppm at C-16 was observed, and combined with the long-range correlation between δ_H 4.82 (d, 7.5 Hz, 1′-H) and δ_C 79.3 (C-16) in the HMBC spectrum, we concluded that the glucopyranosyl group was linked at C-16. Based on these results, the structure of compound **2** can be elucidated as 2β,3β-dihydroxyl-16-*O*-β-D-glucopyranose-24α-al-olean-12-en-28-oic acid.

Compound **2** was tested for its anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* by comparison with a chloramphenicol standard. The results indicated that compound **2** showed the stronger anti-bacterial activity against *Escherichia coli*. On the other hand, when the anti-tumor activity of compound **2** was tested against HL-60, Bel-7402 and HO-8910 cell lines by the SRB method (Skehan et al. 1990), the results showed that it had no effect on the growth of the cell lines at a concentration of 50 μg/ml in contrast to the vincristine sulphate standard.

3. Experimental

3.1. Apparatus

Melting points were determined on a Kofler instrument and are uncorrected. Optical rotations were taken on a Perkin-Elmer 341 polarimeter. IR spectra were determined on a Nicolet NEXUS 670 FT-IR spectrometer. ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were measured on a Mercury Plus-300BB spectrometer using TMS as the internal standard. HRESI MS were recorded on a Bruker APEX II mass spectrometer. EI MS data were obtained on an HP-5988A GC/MS spectrometer. FAB MS data were obtained on a VG-ZAB-HS mass spectrometer (at 70 eV); Silica gel (200–300 mesh) used for column chromatography and silica gel GF_{254} for TLC were made by the Qing-dao Marine Chemical Factory of China. Vincristine sulfate used as a positive control was supplied by Shanghai Hualian Pharmaceutical Company. Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.2. Plant material

The roots of *A. ageratoides* var. *pilosus* Turcz. (Compositae) were collected in Henan province, P. R. China, in September 2002, and identified by Prof. G L Zhang, College of Biological Science, Lanzhou University. A voucher specimen (No. 20020911) was deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

3.3. Extraction and isolation

The air-dried roots of *A. ageratoides var. pilosus* (3.4 kg) were pulverized and extracted with methanol (4 times, 6 days each time) at room temperature. The combined MeOH extract was concentrated under pressure, suspended in water, and then partitioned successively with EtOAc and concentrated to obtain a residue (45 g), which was then subjected to column chromatography over silica gel (500 g, 200–300 mesh) and eluted with a gradient of petroleum ether-EtOAc (20 : 1, 10 : 1, 5 : 1, 3 : 1, 2 : 1, 1 : 1, 0 : 1) to give seven fractions according to their TLC analysis. From the fraction 3 (petroleum ether-EtOAc 5 : 1) compound **3** (5 mg) and **4** (8 mg) were obtained by repeated silica gel column chromatography with petroleum ether-EtOAc (10 : 1) then petroleum ether-acetone (15 : 1). Fraction 5 (petroleum ether-EtOAc 2 : 1) was rechromatographed on a silica gel column with petroleum ether-acetone (8 : 1) then $CHCl_3$-methanol (30 : 1) to give **1** (3 mg), the compound exhibited a purple colour on TLC (silica gel) on heating after spraying with 5% H_2SO_4 in C_2H_5OH. Fraction 7 (petro-

leum ether-EtOAc 0 : 1) was separated on a silica gel column with $CHCl_3$-MeOH (10 : 1) to obtain 2 (20 mg); the compound exhibited a purple colour on TLC (silica gel) on heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.3.1. 2β,3β,16α-Trihydroxyl-24α-al-olean-12-en-28-oic acid (**1**)

White powder, molecular formula: $C_{30}H_{46}O_6$; m.p. 185–186 °C. $[\alpha]_D^{20}$: −20° (c 0.12 Me_2CO). R_f. 0.47 ($CHCl_3$-methanol 8 : 1); IR (ν_{max}^{KBr}, cm^{-1}): 3445 (OH), 1720 (CHO), 1695 (COOH), 1640, 1368, 1300, 1239, 1050, 829; EI-MS m/z (rel int): 502 $[M]^+$ (2), 458 (2), 440 (6), 425 (3), 264 (2), 238 (2), 220 (2), 219 (4), 44 (100); HRESI MS m/z 520.3639 $[M + NH_4]^+$ (calcd. for $C_{30}H_{50}NO_6$ 520.3633). ^1H NMR δ ppm (C_5D_5N, 300 MHz): 9.42 (1 H, s, 24-H), 5.47 (1 H, brs, 12-H), 5.05 (1 H, brs, 16-H), 4.34 (1H, m, 2-H), 3.90 (1 H, d, J = 2.7 Hz, 3-H), 3.43 (1 H, dd, J = 2.7, 10.5 Hz, 18-H), 2.20 (2 H, m, 1-H), 1.47 (1 H, m, 5-H), 1.52 (3 H, s, 23-H), 1.36 (3 H, s, 25-H), 1.64 (3 H, s, 27-H), 0.96 (3H, s, 30-H), 0.85 (3 H, s, 26-H), 0.84 (3 H, s, 29-H); ^{13}C NMR data (Table 1).

3.3.2. 2β,3β-Dihydroxyl-16-O-β-D-glucopyranose-24α-al-olean-12-en-28-oic acid (**2**)

White powder, molecular formula: $C_{36}H_{56}O_{11}$; m.p. 224–225 °C. $[\alpha]_D^{20}$: + 17.0° (c 1.1 Me_2CO). R_f. 0.25 ($CHCl_3$-methanol 5 : 1); IR (ν_{max}^{KBr}, cm^{-1}): 3422 (OH), 1719 (CHO), 1698 (COOH), 1640, 1452, 1386, 1364, 1079, 1038, 714; FAB-MS m/z 687 $[M + Na]^+$, 671 $[M + Li]^+$, 525 [M-glu + Na]$^+$, 509 [M-glu + Li]$^+$; HRESI MS m/z 682.4163 $[M + NH_4]^+$ (calcd. for $C_{36}H_{60}NO_{11}$ 682.4161); ^1H NMR δ ppm (C_5D_5N, 300 MH_z): 9.48 (1 H, s, 24-H), 5.40 (1 H, brs, 12-H), 5.14 (1 H, brs, 16-H), 4.82 (1 H, d, J = 7.5 Hz, 1′-H), 4.30 (1 H, m, 2-H), 3.86 (1 H, d, J = 4.2 Hz, 3-H), 3.38 (1 H, dd, J = 3.9, 10.8 Hz, 18-H), 2.11 (2 H, m, 1-H), 1.49 (1 H, m, 5-H), 1.69 (3 H, s, 27-H), 1.52 (3 H, s, 23-H), 1.33 (3 H, s, 25-H), 0.87 (3 H, s, 30-H), 0.81 (3 H, s, 26-H), 0.67 (3 H, s, 29-H); ^{13}C NMR data (Table).

3.3.3. Friedelinol (**3**)

White solid, molecular formula: $C_{30}H_{52}O$; mp 285–287 °C (Me_2CO). $[\alpha]_D^{20}$: +16.0° (c 0.16 $CHCl_3$). FAB-MS m/z 429 $[M + H]^+$; ^1H NMR δ ppm ($CDCl_3$, 300 MH_z): 3.72 (1 H m, 3-H), 1.16 (3 H, s), 1.00 (3 H, s), 0.98 (3 H, s), 0.97 (3 H, s), 0.95 (3 H, s), 0.94 (3 H, s), 0.93 (3 H, d, J = 6.8 Hz 23-H), 0.85 (3 H, s); ^{13}C NMR data (Table).

3.3.4. Friedelin (**4**)

White needles, molecular formula: $C_{30}H_{50}O$; m.p. 259–260 °C (Me_2CO). $[\alpha]_D^{20}$: −26.1° (c 0.36 $CHCl_3$). EI-MS m/z (rel int): 426 $[M]^+$ (21), 411 $[M\text{-}CH_3]^+$ (5), 273 (23), 205 (22); ^1H NMR δ ppm ($CDCl_3$, 300 MHz): 1.17 (3H, s), 1.05 (3H, s), 1.00 (3H, s), 0.98 (3 H, s), 0.94 (3 H, s), 0.87 (3 H, d, J = 6.8 Hz 23-H), 0.85 (3 H, s), 0.72 (3 H, s); ^{13}C NMR data (Table 1).

3.4. Anti-tumor activity assays

Anti-tumor activity was assayed using the sulforhodamine B (SRB) colorimetric assay. Human leukemia cells, (HL-60), human hepatoma cells (Bel-7402) and human ovarian neoplasm cells (HO-8910) were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, at 37 °C under a humidified atmosphere of 5% CO_2, and dispersed in replicate 96-well plates with 4×103 cells/well for 24 h. Then compound **2** was added. Vincristine sulfate was used as a positive control. After 48 h exposure to the toxins, cell viability was determined by measuring the absorbance at 515 nm with an ELISA reader. Each test was performed in 5 replicates.

3.5. Anti-bacterial assays

The anti-bacterial activity assay was carried out by to the cup-plate method, using chloramphenicol as a positive control. Three strains of bacteria: *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* were cultured in beef broth and incubated at 37 °C for 24 h. After dilution of the beef broth, the three bacteria were cultured in sepearate agar medium dishes, six cups (810 mm) were put onto the dishes, and chloramphenicol and compound **2** (0.2 ml of 100 μg/ml) were respectively added into the cups under aseptic conditions. The dishes were then cultured at 37 °C for 24 h. The zone of inhibition of the growth of bacteria, produced by diffusion of the compounds from the cup into the surrounding medium, was measured to evaluate the anti-bacterial activity. Each test was performed in duplicate.

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