Journal of Asian Natural Products Research, Vol. 8, No. 5, July–August 2006, 425–429

Triterpenes from *Tripterygium wilfordii* Hook

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(Received 1 November 2004; revised 29 December 2004; in final form 8 January 2005)

Two new friedelane-type triterpenes, tripterfrielanons A (**1**) and B (**2**), along with six known triterpenoids, friedelin (**3**), canophyllal (**4**), canophyllalic acid (**5**), 3-oxo-29-hydroxyfriedelane (**6**), wilforlide A (**7**), wilforlide B (**8**), have been isolated from the EtOH extract of the roots of *Tripterygium wilfordii* Hook.f. Compounds **4**, **5**, **6** were isolated for the first time from this plant. The new triterpenes **1** and **2** exhibited mild cytotoxic activity against human Hela cell lines *in vitro*. The assay showed the IC_{50} of **1** and **2** were 8.5 and 25 μg/mL, respectively.

Keywords: *Tripterygium wilfordii Hook.f.*; Celastraceae; Triterpenes; Tripterfrielanons A–B

1. Introduction

Tripterygium wilfordii Hook.*f.*, known as 'Lei-Gong-Teng', is widely distributed in southern China as a Chinese folk medicine. Since the 1960s, it has been found to possess antitumor, anti-inflammatory, anti-HIV and immunosuppressive activities. Lei-Gong-Teng and its preparation have been used for the treatment of various diseases, including dermatitis, rheumatoid arthritis, systemic acne roscoe and nephritis [1,2]. Many pentacyclic triterpenes were previously isolated from this plant. In the course of our continuing search for active constituents, two new friedelane-type triterpenes, tripterfrielanons A (**1**) and B (**2**), along with six known triterpenoids, friedelin (**3**), canophyllal (**4**), canophyllalic acid (**5**), 3-oxo-29-hydroxyfiedelane (**6**) [3], wilforlide A (**7**), wilforlide B (**8**) [4], were isolated from the EtOH extract of the roots of *Tripterygium wilfordii* Hook.f. Compounds **4**, **5**, **6** were isolated for the first time from this plant. Compound **1** is the first report of a friedelane-type triterpenoid with a C-30 formyl function from natural source. The new triterpenes **1** and **2** exhibited mild cytotoxic activity against human Hela cell lines *in vitro*. The assay showed the IC_{50} of **1** and **2** were 8.5 and 25 μg/mL, respectively.

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500172665

2. Results and discussion

Compound **1** was obtained as white amorphous powder and has a molecular formula of $C_{30}H_{48}O_3$ based on HR-ESI-MS at *m/z* 479.3528 $[M + Na]^+$. The IR (KBr) spectrum showed absorption bands at 3442 (—OH), 2933, 2867,1716 (—C═O) cm^{-1}. The ^{13}C and DEPT NMR spectra showed 30 carbon signals including six methyls, twelve methylenes, four methines, six quaternary carbons and two carbonyls (δ_C 213.4, 208.9). The ^1H NMR spectrum showed signals for an aldehyde (δ_H 9.45, s, 1H) and a hydroxymethylene group at δ_H 3.26 (brs, 2H). The HMQC and HMBC analyses revealed that **1** was of the friedelane-type pentacyclic triterpenes [5]. The NMR spectra of **1** were very similar to those of known compound canophyllal (**4**) (see table 1). So the aldehyde group was assigned to C-28, which is coincident with HMBC. In the HMBC experiment, the aldehyde proton signal at δ_H 9.45 (1H, s) was correlated to C-17 (δ_C 48.6s), C-18 (δ_C 35.9d), C-16 (δ_C 28.6t). The difference was the absence of one methyl group and the presence of a hydroxymethylene group (δ_C 74.7t, δ_H 3.26, brs, 2H) in compound **1**. The IR spectrum of **1** showed hydroxyl absorption at 3442 cm^{-1}. A characteristic peak at *m/z* 273 arising from the cleavage of C13–C18 and C14–C15 bonds and a base peak at *m/z* 427 resulting from loss of an aldehyde group (C17–CHO) as seen in a typical friedelan fragmentation pattern led to the conclusion that the CH_2OH group was located to D/E rings. Because of the substitution of hydroxyl group, the carbon signal of C-20 at δ_C 33.7s was shifted downfield from δ_C 28.7 s in comparisons to **4**.

Table 1. The ^{13}C NMR data for compounds **1**, **2**, **4**, **6** ($CDCl_3$, δ in ppm).

Carbon	***1***	***2***	***4***	***6***
1	22.7t	22.0t	22.6t	22.7t
2	41.4t	41.2t	41.9t	41.6d
3	213.4s	212.8s	213.4s	213.7s
4	58.6d	57.9d	58.6d	58.6d
5	42.3s	41.9s	42.3s	42.5s
6	41.9t	41.0t	41.4t	41.9t
7	18.4t	18.0t	18.4t	18.6t
8	53.2d	53.1d	53.2d	53.8d
9	38.0s	37.2s	37.5s	37.8s
10	59.6d	59.2d	59.7d	59.8d
11	35.7t	35.3t	35.8t	36.0t
12	31.0t	29.8t	31.0t	30.9t
13	37.6s	38.0s	38.1s	38.6s
14	39.3s	39.6s	39.1s	40.3s
15	28.6t	32.3t	28.4t	33.5t
16	32.8t	35.5t	32.7t	36.2t
17	48.6s	30.1s	48.1s	31.0s
18	35.9d	41.6d	36.8d	42.2d
19	29.8T	30.3t	35.3t	30.1t
20	33.7s	31.4s	33.8t	33.1s
21	28.0t	27.7t	28.7s	28.2t
22	33.7t	38.8t	32.8t	39.9t
23	7.2q	6.6q	7.2q	7.2q
24	15.0q	14.4q	15.0q	15.0q
25	17.5q	17.6q	17.6q	18.3q
26	20.5q	18.2q	19.2q	18.8q
27	19.2q	20.7q	20.4q	21.1q
28	208.9d	31.8q	209.4d	32.5q
29	24.4q	25.9q	34.9q	75.1t
30	74.7t	74.3t	29.8q	26.2q
HCO-		161.2d		

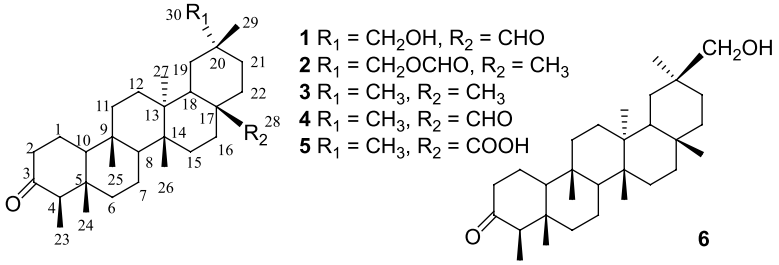

Figure 1. Structures of compounds **1**–**6**.

This suggested that the CH_2OH group was at E ring. The position of hydroxyl group at C-30 was unambiguously elucidated by the ^{1}H NMR analysis. As reported in the literature, there is a minor difference in the coupling pattern of the hydroxymethylene signal at C-29 or C-30 [6]. The supplementary two-proton singlet at δ_H 3.26 (2H, brs) indicated that the hydroxyl group was attached at C-30, which correlated with a hydroxymethyl at C-30 (δ_C 74.7) in the HMQC experiment. Compound **6** with a 29-hydroxy group exhibited two AB doublet signal at δ_H 3.44, 3.39 (each 1H, d, $J = 10.4$ Hz). This was further supported by the HMBC spectrum, which had long-range correlations between H-30 with C-29, C-20, C-19 and C-21 (figure 2). Thus, the structure of **1** was elucidated as 3-oxo-30-hydroxyfriedelan-28-al (figure 1).

Compound **2** was isolated as amorphous powder and showed a molecular formula of $C_{31}H_{50}O_3$ based on HR-ESI-MS at *m/z* 493.3682 $[M + Na]^+$. The IR (KBr) spectrum showed absorption bands at 2929, 2869, 1750, 1731(C═O), 1388, 1162 cm^{-1}. The ^{1}H NMR spectrum showed signals for six methyl singlets (δ_H 0.72, 0.87, 1.03 × 2, 1.07, 1.21), one methyl doublet (δ_H 0.89, d, $J = 6.0$ Hz) and a formate proton singlet at δ_H 8.14 [7]. The ^{13}C and DEPT NMR spectra displayed the presence of seven methyls, twelve methylenes, one of which was oxygenated, five methines, six quaternary carbons, one carbonyl (δ 212.8) and one formyl carbon (δ 161.2) [7]. The EI-MS showed a characteristic peak at *m/z* 273 as seen in a typical friedelane fragmentation pattern. All these evidences suggested that 2 was also a friedelane-type triterpenes. Similar to **1**, the hydroxyl is substituted at C-30. The HMBC experiment supported that the methyl group at C-30 has been oxygenated into a hydroxymethylene unit, indicated by a correlation between H-30 and C-29, C-20 and the formyl carbon. Meanwhile, the correlation from the formyl proton to C-30 supported that the formyl group was linked to C-30 hydroxymethylene (figure 2). Moreover, the correlation between the formyl proton and H-30 confirmed the placement of the —OCHO at C-30 in the

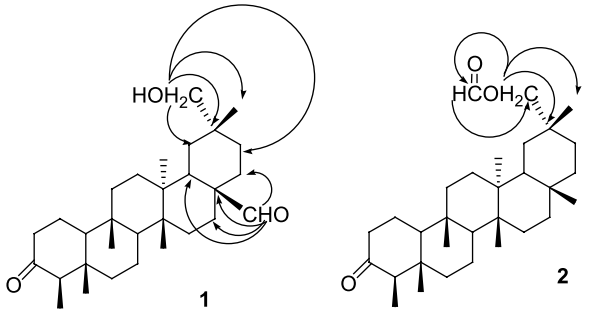

Figure 2. Selective HMBC correlations of **1** and **2**.

^{1}H-^{1}H COSY spectrum. Therefore, the structure of **2** was deduced to be 3-oxo-30-hydroxyfridelane formate (figure 1). So far, friedelane-type triterpene with aldehyde group has been reported [3,5], but this is the first report of a friedelane-type triterpene with a C-30 formyl function from natural sources.

Six known compounds from *Tripterygium wilfordii* Hook.f. were identified by spectroscopic evidence or direct comparison with authentic samples as friedelin (**3**), canophyllal (**4**), canophyllalic acid (**5**), 3-oxo-29-hydroxyfriedelane (**6**) [3], wilforlide A (**7**), wilforlide B (**8**) [4]. Among them, compounds **4**, **5**, **6** were isolated for the first time from this plant.

The new triterpenes **1** and **2** exhibited mild cytotoxic activity against human Hela cell lines *in vitro*. The assay showed the IC_{50} of **1** and **2** were 8.5 and 25 μg/mL, respectively.

3. Experimental

3.1 General experimental procedures

IR spectra were taken on a Nicolet AVATAR-360 spectrophotometer, ν_{max} in cm^{-1}. ^{1}H and ^{13}C NMR spectra were measured with a Bruker AV 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) and AV-500 spectrometer. Chemical shifts (δ) are in ppm relative to TMS as internal standard and coupling constant (*J*) are in Hz. The EI-MS was obtained on VG-Autospec 3000 mass spectrometers. Optical rotation was measured on a Jasco-20 MC Polarimeter. Commerical Silica gel plates (Qing Dao Marine Chemical Group Co.) were used for TLC. The chromatograms were sprayed with 10% H_2SO_4 and heated at 80°C to detect the spots.

3.2 Plant material

The plant sample was collected from Lincang county of Yunnan province in October 2001 and identified by Professor Zhi-Hao Hu of the department of botany, Yunnan University. A voucher specimen (No. 200110) is deposited in the School of Pharmacy, Yunnan University.

3.3 Extraction and isolation

The powdered roots of *Tripterygium wilfordii* Hook.f. (10.0 kg) were repeatedly extracted with EtOH at room temperature. The extract was then concentrated under reduced pressure to give brown syrup, which was partitioned with solvents into petroleum ether-soluble (50 g), EtOAc-soluble (120 g) and *n*-BuOH-soluble (80 g) fractions. The PE-soluble portion was subjected to silica gel column chromatography eluting with PE-EtOAc (10:1–1:1), by which nine fractions (I–IX) were obtained. Fractions II and III were resubmitted to silica gel column chromatography to yield compounds **1** (20 mg), **3** (25 mg), **4** (18 mg), **5** (15 mg) and 6 (21 mg), respectively. The EtOAc-soluble portion was subjected to silica gel column chromatography washing with $CHCl_3$-MeOH (99:1–1:1), by which 15 fractions (I–XV) were obtained. Fraction II (4.0 g) was resubmitted to silica gel column chromatography (petroleum ether-EtOAc (5:1, 3:1, 1:1) to yield **2** (7 mg). Fraction III (8.0 g) was subjected to chromatography over silica gel eluting with $CHCl_3$-MeOH (30:1, 20:1, 10:1) to yield compounds **7** (50 mg) and **8** (20 mg), respectively.

3.4 Cytotoxic activity

Hela (human carcinoma of the cervix) cell lines were grown as a monolayer in Dulbecco's modified eagle's medium, DMEM (Gibco), supplemented with 10% new-born calf serum

(Gibco) and 1% of penicillin-streptomycin mixture (10,000 UI/ml). The cells were maintained at 37°C in 5% CO_2 and 90% humidity. The cytotoxic activitity was assessed using colorimetric MTT reduction assay [8]. The percentage viability was plotted against the compound concentrations, and the 50% cell viability (IC_{50}) was calculated from the curve. All the experiments were repeated three times. The assay showed the IC_{50} of **1** and **2** were 8.5 and 25 μg/mL, respectively.

3.5 Tripterfrielanon A (1)

White amorphous powder. $[\alpha]_D^{24}$ − 10.12 (c 0.35, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3442 (—OH), 2933, 2867,1716 (—C═O). ^1H NMR (δ: ppm, $CDCl_3$): 0.68 (3H, s, 26β-CH_3); 0.71 (3H, s, 24β-CH_3); 0.84 (3H, s, 25β-CH_3); 0.87 (3H, s, $J = 6.7$ Hz, 23β-CH_3); 0.98 (3H, s, 29β-CH_3); 1.08 (3H, s, 27α-CH_3); 3.26 (2H, s, 30-CH_2); 9.45 (1H, s, 28-CHO). ^{13}C NMR data (see table 1). EI MS *m/z*: 456 (7), 438 (10), 427 (100), 273 (25), 109 (60). HR-ESI-MS *m/z*: 479.3528 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3$, 479.3501).

3.6 Tripterfrielanon B (2)

White amorphous powder. $[\alpha]_D^{25}$ -22.8° (c 0.20, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2929, 2869, 1750, 1731(C═O), 1388, 1162. ^1H NMR (δ: ppm, $CDCl_3$): 0.89 (3H, d, $J = 6.0$ Hz, 23β-CH_3); 0.72 (3H, s, 24β-CH_3); 0.87 (3H, s, 25β-CH_3); 1.03 (3H, s, 26β-CH_3); 0.99 (3H, s, 27α-CH_3); 1.21 (3H, s, 28-CH_3); 1.07 (3H, s, 29β-CH_3); 3.84 (2H, s, 30-CH_2); 8.14 (1H, s, HCO-). ^{13}C NMR data (see table 1). EI MS *m/z*: 470 (18), 455 (12), 273 (28), 123 (100), 135 (46), 109 (83), 81 (92), 67. HR-ESI-MS *m/z*: 493.3682 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_3$, 493.3681).

Acknowledgements

The authors thank Mr. Ji-Hua YU for collecting plant material and Mr. Shao-You LI for cytotoxicity assay.

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