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TERPENOIDS FROM *Tripterygium hypoglaucum* AND THEIR ANTI-INFLAMMATORY ACTIVITY

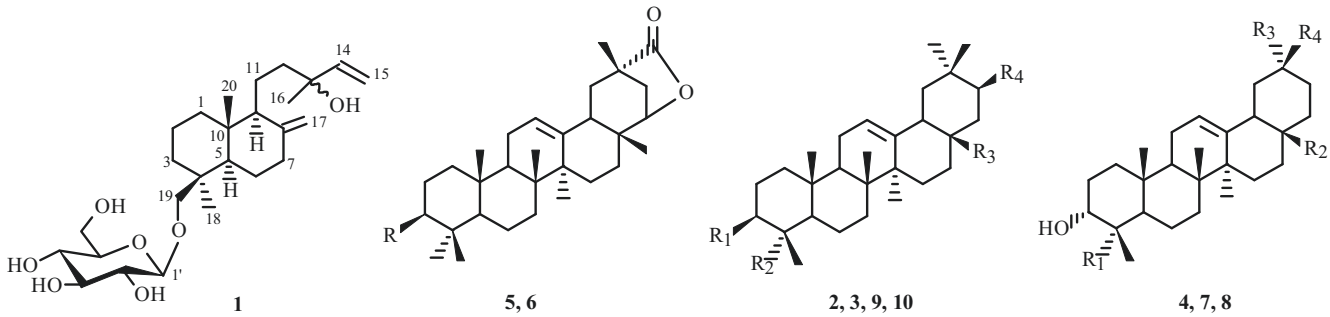
Qun Zhao, Hong-Mei Li, Xuan-Qin Chen, Rong-Tao Li, and Dan Liu*

*A new diterpenoid, 19-*O*-β-D-glucopyranosyl-labda-8(17),14-dien-13-ol (**1**), and nine known triterpenes (**2–10**) were isolated from the aerial parts of* Tripterygium hypoglaucum*. All structures were elucidated by NMR spectroscopic methods. Moreover, the inhibition of superoxide anion generation and elastase release were also examined.*

Keywords: *Tripterygium hypoglaucum*, Celastraceae, diterpenoid, anti-inflammatory.

The genus *Tripterygium* (Celastraceae) includes three species: *Tripterygium wilfordii* Hook. f., *Tripterygium hypoglaucum* (Levl.) Hutch, and *Tripterygium regelii* Sprague et Takeda [1]. The principal chemical constituents of *Tripterygium* are diterpene, triterpene, and sesquiterpene alkaloids, and there are also flavonoids, steroids, tannins, and glucides in it [2]. A number of terpenoids isolated from *Tripterygium* plants, which modulate the production of interferon gamma (IFN-γ), IL-1β, 2, 4, 8, and TNF-α [3–7], exhibit anti-inflammatory and immunosuppressive activities. *T. hypoglaucum* and *T. wilfordii*, two morphologically similar species from this genus, have been used for many years in traditional Chinese medicine for the treatment of swelling, inflammation, cancer, rheumatoid arthritis, and as insecticides [8]. In order to discover more structurally interesting and anti-inflammatory secondary metabolites from the genus *Tripterygium*, a phytochemical investigation on *T. hypoglaucum* was carried out, which led to the isolation of a new diterpenoid and nine known triterpenes. In this paper, we described the isolation and structural determination of the isolates, as well as their anti-inflammatory activity.

Compound **1** was obtained as a colorless oil, and its molecular formula $C_{26}H_{44}O_7$ was established on the basis of HR-EI-MS (*m/z* 468.3108 $[M]^+$; calcd 468.3087), indicating five degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **1** (Table 1) showed characteristic resonances of a sugar and a diterpene moiety. The acid hydrolysis of **1** with 1 M HCl indicated the D-glucose as the sugar residue, which was determined by GC analysis of its corresponding trimethylsilylated L-cysteine derivative.



2: R_1H =O, R_2 = CH_2OH, R_3 = COOH, R_4 = H; **3:** R_1 = OH, R_2 = CH_2OH, R_3 = COOH, R_4 = H
4: R_1 = COOH, R_2 = R_3 = R_4 = CH_3; **5:** RH = O; **6:** R = OH; **7:** R_1 = CH_3, R_2 = COOH, R_3 = CH_2OH, R_4 = CH_3
8: R_1 = CH_3, R_2 = CH_2OH, R_3 = CH_3, R_4 = COOH; **9:** R_1H =O, R_2 = R_3 = CH_3, R_4 = OH
10: R_1 = OH, R_2 = CH_3, R_3 = COOH, R_4 = H

Faculty of Life Science and Technology, Kunming University of Science and Technology, 650500, Kunming, P. R. China, fax: +86 871 65920570, e-mail: liudankm512@163.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2018, pp. 400–402. Original article submitted October 19, 2016.

TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data of Compound **1** (C_5D_5N, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	36.2 t	1.73 (d, J = 10.6)	13	72.7 s	–
		0.98 (dd, J = 2.9, 10.7)	14	147.5 d	6.20 (dd, J = 8.9, 14.4)
2	18.5 t	1.61–1.63 (m)	15	111.1 t	5.58 (dd, J = 1.6, 14.4)
		1.39–1.41 (m)			5.16 (dd, J = 1.6, 8.9)
3	39.3 t	2.33 (d, J = 10.8)	16	28.4 q	1.49 (s)
		0.95 (dd, J = 2.9, 10.7)	17	107.2 t	4.90 (br.s)
4	38.7 s	–			4.81 (br.s)
5	56.6 d	1.12–1.14 (overlapped)	18	28.5 q	1.16 (s)
6	24.8 t	1.66–1.68 (overlapped)	19	72.8 t	4.00 (d, J = 8.0)
		1.73 (d, J = 10.6)			3.89 (d, J = 8.0)
7	38.9 t	0.98 (dd, J = 2.9, 10.7)	20	15.6 q	0.66 (s)
		1.86–1.90 (m)	1r	105.8 d	4.87 (d, J = 6.5)
8	148.9s	–	2r	75.5 d	4.03–4.06 (m)
9	57.7 d	1.56–1.58 (overlapped)	3r	78.5 d	3.96–3.99 (m)
10	40.1 s	–	4r	71.8 d	4.27–4.29 (m)
11	19.6 t	1.77–1.81 (m)	5r	78.7 d	4.24–4.27 (m)
		1.66–1.68 (overlapped)	6r	62.9 t	4.57 (dd, J = 1.8, 9.9)
12	42.4 t	2.01 (td, J = 3.4, 10.4)			4.40 (dd, J = 4.4, 9.9)
		1.49–1.52 (overlapped)			–

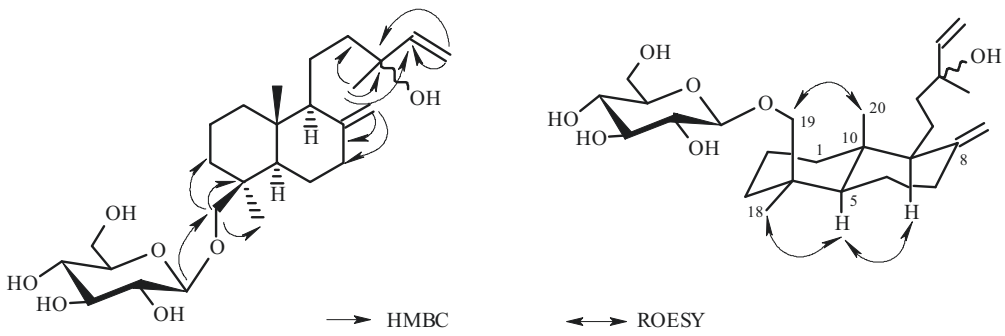


Fig. 1. Key HMBC and ROESY correlations of compound **1**.

The resonance due to the anomeric proton of the glycoside at δ 4.87 (d, J = 6.5 Hz) suggested the presence of a *β*-glucose moiety [9]. Except for the signals of the *β*-D-glucose, three tertiary methyl groups [δ_H 1.49, 1.16, 0.66 (each 3H, s); δ_C 28.4, 28.5, 15.6 (each, q)], ten methylenes including an oxygenated [δ_H 4.00 (1H, d, J = 8.0 Hz, H_a-19), 3.89 (1H, d, J = 8.0 Hz, H_b-19); δ_C 72.8 (t, C-19)], and two unsaturated ones [δ_H 5.58 (1H, dd, J = 14.4, 1.6 Hz, H_a-15), 5.16 (1H, dd, J = 8.9, 1.6 Hz, H_b-15), 4.90 (1H, br.s, H_a-17), 4.81 (1H, br.s, H_b-17); δ_C 111.1 (t, C-15), 107.2 (t, C-17)], two saturated and one olefinic [δ_H 6.20 (1H, dd, J = 14.4, 8.9 Hz, H-14); δ_C 147.5 (d, C-14)] methines, as well as four quaternary carbons including an oxygenated one [δ_C 72.7 (s, C-13)] and an unsaturated one [δ_C 148.9 (s, C-8)], were observed in the ^{1}H and ^{13}C NMR spectra, which were assigned to the labdane diterpene skeleton [10].

Careful comparison of the NMR data of **1** with those of (4*R*,13*S*)-18-*O*-*β*-D-glucopyranosyl-labda-8(17),14-dien-13-ol [11] showed that they were extremely similar, except that the chemical shifts at δ 49.3 (C-5), 79.3 (C-18), and 18.0 (C-19) in the reference compound were changed to δ_C 56.6, 28.5, and 72.8, respectively, in **1**. These differences suggested that the *β*-D-glucose was attached to C-19 in **1** rather than C-18. The HMBC correlations (Fig. 1) of **1** observed between the anomeric proton (H-1′) and the oxygenated methylene carbon (C-19), and the ROESY correlations (Fig. 1) of H_3-20/H_2-19, H-5/H_3-18, and H-5/H-9 confirmed the location of the oxygenated methylene at C-19. Based on these data, the structure of compound **1** was determined as 19-*O*-*β*-D-glucopyranosyl-labda-8(17),14-dien-13-ol.

By comparison of the NMR data with the literature, nine known compounds were identified as 23-hydroxy-3-oxo-olean-12-en-28-oic acid (**2**) [12], 3,23-hydroxy-olean-12-en-28-oic acid (**3**) [13], *α*-boswellic acid (**4**) [14], wilforlide B (**5**) [15], wilforlide A (**6**) [15], olean-12-en-28-oic acid (**7**) [16], 3*α*,28-dihydroxy-olean-12-en-29-oic acid (**8**) [6], 3-oxo-21-hydroxy-olean-12-ene (**9**) [17], and oleanolic acid (**10**) [18].

A target assay based on effects against superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB was carried out for compounds **1**–**10**. The results showed that compounds **2** and **3** indicated anti-inflammatory activity against superoxide anion generation and elastase release (IC_{50} = 1.53 and 1.93 μM for **2**; IC_{50} = 4.39 and 1.92 μM for **3**).

EXPERIMENTAL

Genaral. Column chromatography (CC): silica gel (200–300 or 100–200 mesh, Qingdao Marine Chemical Factory, P. R. China), MCI gel (75–150 μm, Mitsubishi, Japan), Lichroprep RP-18 (40–63 μm, Merk, Germany) and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden). Thin-layer chromatography (TLC): silical gel GF_{254} plates (Qingdao Marine Chemical Factory, P. R. China), visualization by spraying with 10% H_2SO_4–EtOH, followed by heating on a hot plate. Semipreparative HPLC: Agilent 1200 system with a Zorbax SB-C_{18} column (5 μm, 9.4 × 250 mm). Optical rotations: Jasco DIP-370 digital polarimeter. 1D and 2D NMR Spectra: Bruker AM-400, DRX-500, and Avance III 600 spectrometers with TMS as the internal standard. EI-MS and HR-EI-MS Spectra: VG Auto Spec-3000 spectrometer.

Plant Material. The aerial parts of *T. hypoglaucum* were collected in Cang Mountain, Dali, Yunnan Province, China, in September 2011. The sample was identified by Dr. Yong-Peng Ma, Kunming Institute of Botany, Chinese Academy of Science, and a voucher specimen (KMUST 2011092601) has been deposited in our laboratory.

Extraction and Isolation. Air-dried and powdered aerial parts of *T. hypoglaucum* (10 kg) were extracted with 80% acetone (3 × 15 L, 1 d, each) at room temperature and then concentrated *in vacuo* to give an extract, which was suspended in H_2O and then successively extracted with EtOAc (4 × 4 L) and *n*-BuOH (3 × 4 L). The EtOAc extract (460 g) was subjected to silica gel CC eluting with $CHCl_3$–acetone (10:0, 9:1, 8:2, 7:3, 6:4, v/v) to give five fractions (Frs. A–E). Fraction B (21.5 g) was chromatographed on a MCI column (MeOH–H_2O, 90% and 100%) to obtain five subfractions (Subfrs. B1–B5). Subfraction B1 (1.11 g) was subjected to silica gel column chromatography using petroleum ether–acetic ester to give **1** (12.0 mg). Subfraction B2 (5.5 g) was applied on a Lichroprep RP-18 gel column, eluting with MeOH–H_2O (50, 70, 90 and 100%) to give four subfractions (Subfrs. B2.1–B2.4). Subfraction B2.2 (1.8 g) was subjected to silica gel column chromatography using $CHCl_3$–MeOH (300:1) and purified by Sephadex LH-20 column to obtain compound **2** (5.0 mg). Fraction C (19 g) was chromatographed on a Sephadex LH-20 column (60 × 600 mm), using MeOH–H_2O (30, 60, 90, and 100%) as eluent to give five subfractions (Subfrs. C1–C5). Subfraction C4 (2.4 g) was subjected to silica gel CC with $CHCl_3$–MeOH (1:0, 100:1, 25:1, 10:1, 1:1, 0:1) to afford six subfractions (Subfrs. C4.1–C4.6). Subfraction C4.1 (1.5 g) was subjected to silica gel CC, eluting with $CHCl_3$–MeOH (1:0–0:1), to yield subfractions C4.1.1–C4.1.5. Subfraction C4.1.4 (135.0 mg) was applied on a MPLC RP-18 (3 mL/min, MeOH–H_2O, 60, 80%) to give compounds **6** (10.0 mg), **7** (5.8 mg), and **8** (6.0 mg). Subfraction C4.2 (1.12 g) was subjected to silica gel CC with petroleum ether–acetone (4:1), then purified by another silica gel column and eluted using petroleum ether–EtOAc (8:1) to yield compounds **5** (7.0 mg) and **10** (15.0 mg). Subfraction C4.3 (0.87 g) was applied on a MPLC RP-18 column (3 mL/min, MeOH–H_2O, 65%) to give four subfractions (Subfr. C4.3.1–C4.3.4). Subfraction C4.3.1 (338 mg) was separated on a silica gel column, eluting with petroleum ether–acetone (6:1), to give compounds **3** (5.0 mg), **4** (8.0 mg), and **9** (10.0 mg).

Acid Hydrolysis of 1. Compound **1** (2.0 mg) was hydrolyzed with 1 M HCl (0.5 mL) in a screw-capped vial for 3 h at 90°C. The mixture was evaporated to dryness under vacuum, and then the residue was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was collected and neutralized by addition of Amberlite IRA400 (OH-form) and filtered. The filtrate was dried *in vacuo*, then dissolved in 0.2 mL of pyridine containing L-cysteine methyl ester (10 mg/mL) and reacted at 60°C for 1 h. To this mixture a solution (0.2 mL) of trimethylsilylimidazole in pyridine (10 mg/mL) was added, and it was heated at 60°C for 1 h [19]. The final mixture was directly analyzed by GC [30QC2/AC-5 quartz capillary column (30 m × 0.32 mm) under the following conditions: column temperature: 180°C/280°C; programmed increase 3°C /min; carrier gas: N_2 (1 mL/min); injection and detector temperature: 250°C; injection volume: 4 μL; split ratio: 1:50]. The standard D-glucose was prepared following the same procedure. Under these conditions, the retention time for D-glucose was 18.29 min; the hydrolysate had the same retention time with it.

The preparation of human neutrophils and measurement of superoxide anion generation and elastase release were carried out following the method in the literature [20].

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