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A NEW ABIETANE DITERPENOID FROM *Isodon lophanthoides* var. *graciliflorus*

Jing-jin Wang,[1,2] Lai-lai Wong,[3] Ke-Rong Jiang,[1] Xian Li,[4] Wen-fei Chen,[4,5*] and Shu-de Li[1*]

*A new abietane diterpenoid, graciliflora A (**1**), was isolated from the aerial parts of* Isodon lophanthoides *var*. graciliflorus*. Its structure was elucidated by spectroscopic methods, including extensive 1D and 2D NMR analysis. Compound **1** exhibited good anti-inflammatory and cytotoxic activities.*

Keywords: *Isodon lophanthoides* var. *graciliflorus*, abietane diterpenoid, anti-inflammatory activity, cytotoxic activity.

The aerial parts of *Isodon lophanthoides* are empirically employed as antimalarial and anti-inflammatory agents and also for the treatment of enteritis and jaundice [1]. *Isodon lophanthoides* var. *graciliflorus* (Benth.) H. Hara is a variety of *I. lophanthoides*, which is a perennial herb distributed in Fujian, Guangdong, and Jiangxi Provinces of China [2]. It is known in China by the name "*tiancao*" and has been commercially farmed as a source of 'Xihuangcao,' a Chinese folk medicine for the treatment of acute icterohepatitis, cholecystitis, and enteritis as well as an herb for health promoting beverages such as tea and instant granules [3]. The water extract of *I. lophanthoides* var. *graciliflorus* showed stronger anti-inflammatory activity [4]. It has been demonstrated that the predominant constituents of this plant are abietane diterpenoids, which exhibit remarkable cytotoxic and anti-inflammatory activities [5, 6]. In our search for anti-inflammatory and cytotoxic bioactive diterpenoids from this plant, investigation of the chemical constituents of *I. lophanthoides* var. *graciliflorus* collected in Guangzhou led to the isolation of a new abietane diterpenoid, graciliflora A (**1**). The present paper deals with the isolation, structure elucidation, anti-inflammatory evaluation, and cytotoxic evaluation of the new compound.

Graciliflora A (**1**) was isolated as brown solid and yielded a pseudomolecular ion peak in the negative HR-ESI-MS at *m*/*z* 383.2230 $[M - H]^-$, indicative of the molecular formula $C_{24}H_{32}O_4$ with nine degrees of unsaturation. The IR showed the characteristic absorption bands of hydroxy (3419 cm^{-1}), aromatic ring (1616, 1470 cm^{-1}), and α,β-unsaturated carbonyl group (1654 cm^{-1}). Identifiable from the ^{13}C NMR spectroscopic data for **1** (Table 1) were resonances consistent with two trisubstituted double bonds [δ 120.0 (d), 165.5 (s), and 113.5 (d), 148.3 (s)], six aromatic carbons [δ 113.6 (d), 123.5 (s), 128.4 (s), 137.0 (s), 144.3 (s), 146.3 (s)], and a carbonyl carbon [δ 201.4 (s). In the absence of any other sp or sp^2 carbon, the structure of **1** must be tricyclic. A careful analysis of the HSQC, ^1H–^1H COSY, and HMBC spectroscopic data suggested that **1** was an abietane diterpenoid, and its PMR and ^{13}C NMR data exhibited great similarity to those of the known compound isolophanthin D (**2**) except for an additional double band at the C(7)=C(1′) position in **1** [7]. Key HMBC correlations (Fig. 1) observed from H-1′ (δ 6.53) to C-6 (δ 120.0) and C-8 (δ 123.5) and from H-3′ (δ 2.29) to C-1′ (δ 113.5) and C-2′ (δ 201.4) in **1** confirmed that an acetonylidene group was linked to C-7 position. The acetonylidene group showed signals at δ 2.29 (3H, s, CH_3-3′), 6.53 (1H, s, H-1′), and 32.5 (q, C-3′), 113.5 (d, C-1′), and 201.4 (s, C-2′). The relative stereochemistry of **1** was determined through analysis of correlations observed in the ROESY spectrum as shown in a computer-generated 3D drawing (Fig. 1).

1) Department of Biochemistry and Molecular Biology, School of Basic Medicine, Kunming Medical University, 650500, Kunming, Yunnan, P. R. China, e-mail: shudeli006@163.com; 2) The Second Faculty of Clinical Medicine, Kunming Medical University, 650500, Kunming, Yunnan, P. R. China, e-mail: 1059219327@qq.com; 3) School of Chinese Medicine, Hong Kong Baptist University, 999077, Hong Kong SAR, P. R. China; 4) School of Pharmaceutical Science and Yunnan Key Laboratory of Pharmacology of Natural Products, Kunming Medical University, 650500, Kunming, Yunnan, P. R. China, e-mail: chenwenfeify@qq.com; 5) Affiliated Hospital of Panzhihua University, 617000, Panzhihua, Sichuan, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2021, pp. 401–403. Original article submitted June 14, 2020.

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TABLE 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of Compound **1** (CD_3OD, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1β	3.46 (1H, ddd, J = 8.34, 4.62, 4.44)	36.1 (CH_2)	12	–	146.3 (C)
1α	1.34 (1H, m)		13	–	128.4 (C)
2β	2.00 (1H, m)	19.9 (CH_2)	14	7.22 (1H, s)	113.6 (CH)
2α	1.63 (1H, m)		15	–	81.3 (C)
3β	1.68 (1H, m)	41.9 (CH_2)	16	1.62 (3H, s)	26.7 (CH_3)
3α	1.40 (1H, dt, J = 12.6, 3.7)		17	1.66 (3H, s)	26.4 (CH_3)
4	–	39.1 (C)	18	1.32 (3H, s)	33.9 (CH_3)
5	–	165.5 (C)	19	1.38 (3H, s)	30.8 (CH_3)
6	8.15 (1H, s)	120.0 (CH)	20	1.56 (3H, s)	25.5 (CH_3)
7	–	148.3 (C)	1′	6.53 (1H, s)	113.5 (CH)
8	–	123.5 (C)	2′	–	201.4 (C)
9	–	137.0 (C)	3′	2.29 (3H, s)	32.5 (CH_3)
10	–	42.9 (C)	MeO-15	3.24 (3H, s)	51.3 (CH_3)
11	–	144.3 (C)			

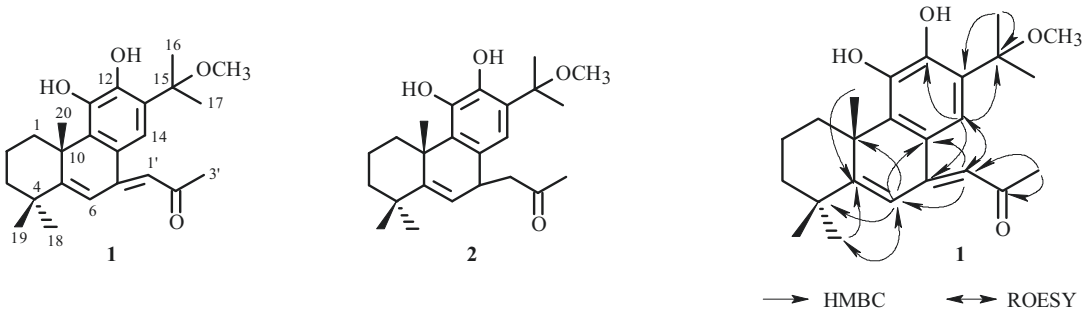

Fig. 1. Structures of compounds **1** and **2**, HMBC and ROESY correlations of **1**.

The observation of the ROESY correlation from H-14 to H-1′ suggested that the C(7)=C(1′) bond was in the *E*-configuration. Accordingly, the structure of compound **1** was established as (*E*)-7-acetonylidene-11,12-dihydroxy-15-methoxy-abieta-5,8,11,13-tetraene and named graciliflora A.

Compound **1** was assayed for TNF-α inhibitory effect on LPS-induced RAW264.7 cells with dexamethasone as the positive control. Compared with dexamethasone (IC_{50} = 509.60 ± 26.53 μM), compound **1** showed middle activity with the IC_{50} value of 36.83 ± 2.42 μM. The influence of compound **1** on HCT116 cell viability was evaluated by the CCK-8 assay. Compound **1** (5.204, 52.04 μM) supressed HCT116 cell viability with inhibitory rates of 2.83% and 87.25%, respectively. The data indicated that compound **1** could inhibit HCT116 cell proliferation.

EXPERIMENTAL

General. NMR spectra were recorded on a Bruker AM-400 spectrometer, a Bruker DRX-500 spectrometer, and a Bruker Avance III-600 spectrometer in CD_3OD with TMS as internal standard. Mass spectra were taken on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. IR spectra were scanned using a Bio-Rad Tensor27 spectrometer as KBr pellets. Ultraviolet absorption spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. The positive control was dexamethasone (water-soluble; Sigma Chemical Co., St. Louis, Missouri). The cell counting kit-8 was purchased from Yeasen Biotech Co., Ltd. Materials for column chromatography were silica gel (200–300 mesh; Qingdao Marine Chemical Inc.) and Sephadex LH-20 (40–70 μm; Merck Co., Ltd.). Spots were visualized under UV light (254 nm) or by spraying with 10% H_2SO_4 in 95% EtOH (v/v) followed by heating.

Plant Material. The aerial parts of *I. lophanthoides* var. *graciliflorus* (Benth.) H. Hara were collected from Guangzhou City, Guangdong Province, P. R. China, in May 2015. Identification of the plant was performed by Dr. Lai-lai Wong of Hong Kong Baptist University. A voucher specimen (LLW 0028) was deposited in Hong Kong Baptist University.

Extraction and Isolation. Powder (12 kg) of the dried aerial parts was extracted with acetone (30 L × 4) at room temperature. Filtration and condensation of the solution to dryness under vacuum yielded an extract (400 g). The extract was dissolved in distilled water (3 L) and then fractionated with ethyl acetate and *n*-butyl alcohol. The ethyl acetate part (354 g) was subjected to silica gel column chromatograph (PE–CP, 100:0–0:100) to give 10 fractions (A_1–A_{10}). Fraction A_4 (25.0 g) was removed of pigment by MCI gel, then separated by silica gel CC (PE–CP, 100:0 to 0:100) to get four subfractions ($A_{4\text{-}1}$–$A_{4\text{-}4}$). Subfraction $A_{4\text{-}1}$ was subjected to silica gel CC (PE–EtOAc, 30:1–1:1) to give three subfractions ($A_{4\text{-}1\text{-}1}$–$A_{4\text{-}1\text{-}3}$). Subfraction $A_{4\text{-}1\text{-}3}$ was subjected to silica gel CC (PE– EtOAc, 5:1), then purified by Sephadex LH-20 (MeOH–$CHCl_3$, 1:1) and semipreparative HPLC (MeOH–H_2O, 75:25), followed by recrystallization to get compound **1** (6.8 mg).

Graciliflora A (1). Brown solid, mp 108–112°C; $[\alpha]_D^{25}$ +84.4° (*c* 0.12, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 205 (4.35). IR (KBr, ν_{max}, cm^{-1}): 3419 (OH), 1654 (C=O), 1616, 1470 (Ar). HR-ESI-MS *m*/*z* 383.2230 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$, 383.2228). ^1H NMR (600 MHz, CD_3OD, δ, ppm) and ^{13}C NMR (150 MHz, CD_3OD, δ, ppm), see Table 1.

Anti-inflammatory Assay. The anti-inflammatory activities of compound **1** was evaluated on a cell model of inflammation induced by lipopolysaccharide. RAW264.7 cells in the logarithmic growth stage at a concentration of 1×10^5 cells/mL were inoculated into 96-well plate with 100 μL per well. After cell adherence, 1 μg/mL of lipopolysaccharide (LPS) and various gradient concentrations (200, 40, 8, 1.6, 0.32 μg/mL) of the compound were added in triplicate. Cell culture medium was used as the blank control and dexamethasone was used as the positive control. After 24 h of culture, the cell culture supernatants were collected, and the levels of TNF-α were detected by ELISA kit. The 50% inhibitory concentration (IC_{50}) against the production of TNF-α was calculated.

Cell Counting Kit-8 (CCK-8) Assay. Cell viability was evaluated by the CCK-8 assay. HCT116 cells (5000 cells/well) were seeded on 96-well plates. After cell adherence, compound **1** in various gradient concentrations (5.204, 52.04 μM) were added. After 48 h of culture, 10 μL CCK-8 reagent was added and the whole allowed to react for 2 h. The OD value at 450 nm was measured by a Thermo Multiskan Spectrum plate reader.

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REFERENCES

1. Kunming Institute of Botany, Chinese Academy of Sciences, *Flora Yunnanica Tomus* (in Chinese), Science Press, Beijing, 1977, 758 pp.
2. Z. Y. Wu and P. H. Raven, *Flora of China* (in English), **17**, Science Press, Beijing, 1994, 269 pp.
3. L. Lin, Y. Dong, B. Yang, and M. Zhao, *Comb. Chem. High. Throughput. Screen*, **14**, 720 (2011).
4. Y. H. Kuang, Q. Lin, L. Huang, Y. Li, and D. Q. Wang, *Tianjin J. Trad. Chin. Med.*, **30** (8), 495 (2013).
5. W. T. Zhou, H. H. Xie, P. Wu, and X. Y. Wei, *Food Chem.*, **136**, 1110 (2013).
6. W. F. Chen, L. L. Wong, X. Zhang, F. X. Zhou, F. Xia, L. P. Tang, and X. Li, *Chem. Biodiv.*, **6**, e1900206 (2019).
7. L. B. Yang, L. Li, S. X. Huang, J. X. Pu, Y. Zhao, Y. B. Ma, J. J. Chen, C. H. Leng, Z. M. Tao, and H. D. Sun, *Chem. Pharm. Bull.*, **59** (9), 1102 (2011).