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LC-MS guided isolation of gracilistones A and B, a pair of diastereomeric sesquiterpenoids with an unusual tetrahydrofuran-fused tricyclic skeleton from *Acanthopanax gracilistylus* and their potential anti-inflammatory activities

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ABSTRACT

Gracilistones A (**1**) and B (**2**), two new eudesmane-type sesquiterpenoids with an unusual tetrahydrofuran-fused 6/6/5 tricyclic ring system, were obtained from *Acanthopanax gracilistylus* under the guidance of LC-MS investigation. Their structures and absolute configurations were assigned by extensive spectroscopic analyses and quantum calculation methods. Compounds **1** and **2** showed potent inhibitory activity against LPS-induced nitric oxide production in RAW 264.7 macrophages, compared with the positive control L-NMMA. In addition, compounds **1** and **2** were also evaluated for their antioxidant (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) and xanthine oxidase (XO) inhibitory activities, and they exhibited weak inhibitory effects at 100 μM.

1. Introduction

Eudesmane-type sesquiterpenoids are widespread in nature, and more than 2000 representatives of this class of compounds have so far been obtained in plants [1], fungi [2] and insects [3]. Generally, the natural eudesmane-type sesquiterpenoids have an oxygenated structure, and alcohols, aldehydes, ketones, carboxyl acids and lactones are major class of the sesquiterpenoids [1]. Nevertheless, eudesmane epoxides, especially to one with furan or tetrahydrofuran-fused epoxy pattern, were rare and, hitherto only about 50 representatives of furan or tetrahydrofuran-fused epoxides have been obtained from natural source. Structurally, furan or tetrahydrofuran-fused eudesmane sesquiterpenoids can be mainly classified into three subtypes: (1) 12,8-epoxyeudesmane (*ca.* 30 compounds, *e.g.* atractylon and 3*β*-hydroxyatractylon [4]), (2) 4,14-epoxyeudesmane [13 compounds, *e.g.* 4*α*, l4-epoxy-l*α*-hydroxy-5,10-bis-epi-eudesma-11(13)-ene [5]], (3) 1,4-epoxyeudesmane (7 compounds, *e.g.* 6*β*-cinnamoyloxy-5,10-bis-epi-eudesmane-l*β*,4*β*-epoxide [6]). However, up to now, eudesmane epoxides with a furan or tetrahydrofuran ring in the position 6 (13) have been rarely reported.

Acanthopanax gracilistylus W. W. Smith, belonging to the family Araliaceae, is a useful plant widely distributed in southern China. The root bark of *Acanthopanax gracilistylus* (Acanthopanacis Cortex) is a well-known traditional Chinese medicine (TCM) documented in Chinese Pharmacopoeia (2015 edition). Particularly, Acanthopanacis Cortex is famous with the Chinese name of "Wu-Jia-Pi" and widely used for anti-inflammatory, anti-allergic and diuretic purposes in China [7–10]. The earliest usage of Acanthopanacis Cortex can be traced back to "Shen-Nong-Ben-Cao-Jing", the first Chinese Materia Medica, which probably appeared at the end of the third century [7].

Previous phytochemical investigation on the root bark of *A. gracilistylus* resulted in the isolation of around 40 compounds, including 30 diterpenoids [7], 2 triterpenoids [7,11] and several steroids [12,13]. To the best of our knowledge, sesquiterpenoids have not been obtained from this plant so far.

Currently, liquid chromatography linked with mass spectrometry

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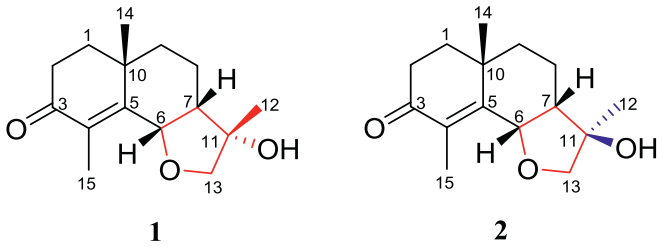

Fig. 1. Structures of gracilistones A (**1**) and B (**2**).

(LC-MS) has become a routine method in many areas of analytical chemistry. The Shimadzu UFLC-MS-IT-TOF apparatus equipped with an electrospray ionization source coupled to ion-trap and time-of-flight mass analysers (ESI-IT-TOF) enables high-resolution mass spectra in both positive and negative modes and, thus, is effective for characterizing trace components in a complex mixture of natural products [14,15].

During our ongoing search for structurally intriguing and biologically valuable natural products from TCMs, LC-MS method was applied to investigate the metabolites from Acanthopanacis Cortex, which led to the isolation of a pair of diastereomeric sesquiterpenoids, gracilistones A–B (**1**–**2**) (Fig. 1), with an unusual tetrahydrofuran-fused tricyclic skeleton. Herein, we report their isolation, structural elucidation, and bioactivities (anti-inflammatory, antioxidant and xanthine oxidase inhibitory activities).

2. Experimental

2.1. General experimental procedures

NMR spectra were obtained on Bruker AVANCE III-600 spectrometer (Bruker, Bremerhaven, Germany). The LC-MS analyses were performed on an LC-MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). UV spectra were recorded on a Shimadzu UV2401PC spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotation measurements were conducted on a Jasco model 1020 digital polarimeter (Horiba, Tokyo, Japan). ECD spectra were recorded on a Chirascan spectrometer (Applied Photophysics, Leatherhead, UK). The semi-preparative HPLC was performed on a CXTH LC-3000 HPLC system (Beijing Chuangxintongheng Science and Technology Co., Ltd., Beijing, China) using a YMC-Pack ODS-A C_{18} (5 μm, 10 × 250 mm) column (YMC Co., Ltd., Kyoto, Japan).

DPPH, ABTS and Xanthine oxidase were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other reagents used in the study were of analytical grade and purchased from Aladdin (Aladdin Reagent Co., Shanghai, China).

2.2. Plant material

The root barks of *A. gracilistylus* were collected from Qichun County, Hubei Province, China and identified by Dr. Wei Wang (Shaanxi University of Chinese Medicine, China). A voucher specimen (No. 20170201) was deposited at the Laboratory of Shaanxi Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Shaanxi University of Chinese Medicine.

2.3. Physical and spectroscopic data of compounds *1* and *2*

Gracilistone A (**1**): colorless oil; $[\alpha]_D^{23}$ −29.8 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 201 (3.32), 245 (3.40); HR-ESI-MS (+) *m/z* 251.1641 $[M + H]^+$ ($C_{15}H_{20}O_3$, calcd. for 251.1642). ^{1}H and ^{13}C NMR data see Table 1.

Gracilistone B (**2**): colorless oil; $[\alpha]_D^{23}$ 83.3 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 200 (3.58), 245 (3.86); HR-ESI-MS (+) *m/z* 251.1637 $[M + H]^+$ ($C_{15}H_{20}O_3$, calcd. for 251.1642). ^{1}H and ^{13}C NMR data see Table 1.

Table 1
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** and **2** in CD_3OD.

NO.	**1**		**2**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1a	1.88, ddd (15.4, 14.3, 5.0)	38.9	1.84, m[a]	39.0
1b	1.76, ddd (15.4, 5.5, 2.1)		1.73, ddd (13.4, 5.8, 2.4)	
2a	2.74, ddd (18.7, 14.3, 5.5)	34.9	2.71, ddd (17.1, 14.8, 5.5)	34.9
2b	2.43, ddd (18.7, 5.0, 2.1)		2.40, ddd (17.1, 5.0, 2.4)	
3		202.0		201.9
4		135.2		135.5
5		158.6		158.3
6	4.89, d (5.0)	77.8	5.22, d (4.5)	77.5
7	1.97, m	51.3	1.95, ddd (11.8, 6.1, 4.5)	52.4
8a	1.81, m	19.0	1.62, m[a]	20.7
8b	1.68, ddd (14.5, 3.2, 3.0)		1.43, m	
9a	1.67, m[a]	40.0	1.61, m[a]	40.4
9b	1.37, ddd (14.5, 3.2, 3.0)		1.34 m[a]	
10		35.4		35.4
11		79.9		82.2
12	1.49, s	28.2	1.33, s	20.5
13a	3.85, d (8.1)	77.9	3.87, d (9.0)	78.3
13b	3.62, d (8.1)		3.68, d (9.0)	
14	1.29, s	23.6	1.25, s	23.5
15	1.83, s	10.7	1.84, s	10.7

[a] Overlapped.

2.4. Computational details

The ECD calculations for compounds **1** and **2** were performed using Gaussian 09 program. Conformers were generated by MMFF94s force field, and each conformer was optimized with the HF/6-311G(d, p) method, and further optimized by the DFT method at the B3LYP/6-311G(2d, p) level in Gaussian 09 program package. Frequency calculations were also performed at the same level to confirm that each optimized conformer was true minimum. The energies, oscillator strengths, and rotational strengths (velocity) of the first 30 electronic excitations were calculated using the TDDFT methodology at the B3LYP/6–311 + G(2d, p) level in methanol. Solvent effects were taken into consideration using the SCRF method with the IEFPCM model. The ECD spectra were simulated by the SpecDis program. To obtain the final conformationally averaged data, the simulated spectra of the predominant conformers were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG).

To further confirm the ECD assignments, specific rotations were calculated in the B3LYP/aug-cc-pVDZ-SCRF (MeOH) level based on the above DFT optimized geometries. The calculated specific optical rotation data of these conformers were averaged according to the Boltzmann distribution theory and their ΔG.

2.5. Anti-inflammatory bioassays

2.5.1. Cell culture and cell viability assay

Mouse macrophages (RAW264.7 cells) were cultured in RPMI 1640 containing 10% FBS, 100 μg/mL streptomycin and 100 units/mL penicillin at 5% CO_2 for 24 h. Cells were then seeded into 96-well plate at a density of 2.5 × 10^5/mL and incubated with serum-free media in the presence of different concentrations of samples. After incubation for 24 h, 15 μL of MTT (0.25 mg/mL final concentration) was added and incubation was continued for another 4 h at 5% CO_2 and 37 °C. The formazan crystals were then solubilized in DMSO (150 μL) and the absorbance was measured at 490 nm by using UV microplate reader.

2.5.2. Assessment of cellular NO production

RAW264.7 cells were cultured at a density of 2.5 × 10^5/mL in a 96-