SHORT REPORT

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A New *ent*-Atisane Diterpenoid from the Stems of *Euphorbia royleana*

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Abstract: A new *ent*-atisane diterpenoid, named euphrosanoid A (**1**), along with five known analogues (**2**–**6**) were isolated from the stems of *Euphorbia royleana*. The structure of new compound **1** was determined by extensive spectroscopic data, especially 1D and 2D NMR experiments, and the absolute configuration of **1** was determined by comparison of experimental and calculated electronic circular dichroism (ECD) data. All isolated compounds were evaluated for their cytotoxic activities, and compounds **1** and **5** showed weak cytotoxicities against HCT-15 cancer cells with IC_{50} values of 62.98 ± 6.27 and 44.35 ± 3.66 μM, respectively.

Keywords: *Euphorbia royleana*; cytotoxic activity; *ent*-atisane diterpenoid.

1. Plant Source

The stems of *Euphorbia royleana* were collected from Guangdong Province, China, in August 2020. The identification of this plant were carried out by Prof. Dr. Jianyong Zhu from Shanghai University of Traditional Chinese Medicine and, by Prof. Dr. Haibo Huang from School of Chinese Materia Medica, Guangzhou University of Chinese Medicine. The voucher specimens were deposited in the Herbairum of the universities and coded as 202008-ERL and GUCM 272964, respectively.

2. Previous Studies

Euphorbia royleana Boiss. (Euphorbiaceae), known as “Ba Wang Bian” in traditional Chinese medicine (TCM), is a thorny succulent shrub [1]. This plant grows widely in Sichuan, Guangxi and Yunnan provinces of China, and has long been used to treat inflammation and rheumatic pain [1]. Previous phytochemical studies of this plant showed that diterpenoids were the main metabolites, including polycyclic diterpenoids (atisanes, kauranes and abietanes, etc.) and macrocyclic diterpenoids (cembranes, ingenanes and ingols) [2-5]. Some of these diterpenoids possessed a broad range of biological activities such as multidrug resistance reversal and NO inhibitory activities [2,3].

3. Present Study

The ethanol extract of the EtOAc-soluble fraction of *E. royleana* was separated by repeated column chromatography (CC) via silica gel and LH-20 gel to give six diterpenoids (**1**–**6**) (Figure 1), including one new *ent*-atisane diterpenoid and five known compounds.

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*Euphrosanoid A (**1**):* white amorphous powder, $[\alpha]^{25}_{D}$ +53.5 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3484, 2948, 1746, 1715, 1438, 1371, 1229, 1055, 754 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 397.1996 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_5Na^+$, 397.1985).

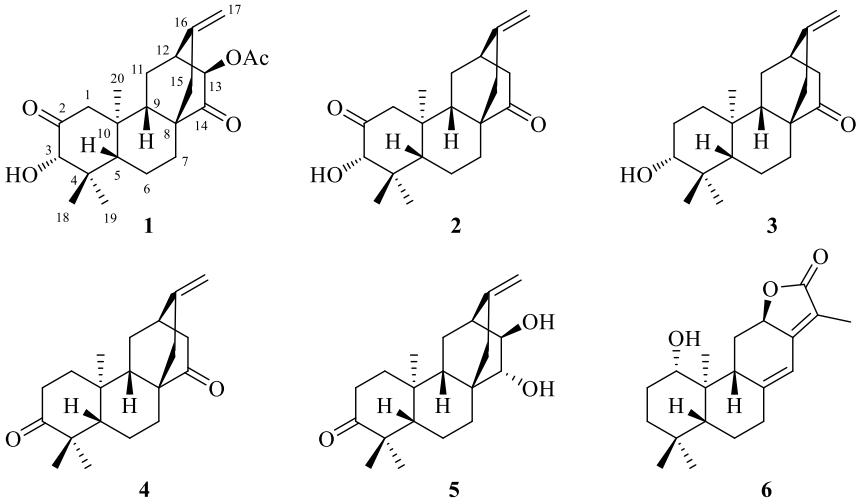

Figure 1. Structures of compounds **1**–**6**

Euphrosanoid A (**1**), was isolated as white amorphous powder with the molecular formula $C_{22}H_{30}O_5$, as established by the HRESIMS data ($[M + Na]^+$ *m/z* 397.1996, calcd 397.1985), requiring eight degrees of unsaturation. The IR spectrum displayed characteristic absorption bands at 3484 (hydroxy group), 1746 and 1715 (carbonyl groups) cm^{-1}. The ^{1}H NMR data (Table 1) displayed proton signals attributed to four methyl groups [δ_H 0.64 (3H, s), 0.68 (3H, s), 1.17 (3H, s) and 2.07 (3H, s)], two oxygenated methines [δ_H 3.88 (1H, s) and 4.95 (1H, d, J = 2.9 Hz)] and one olefinic methylene [δ_H 4.81 (1H, s) and 4.92 (1H, s)]. The ^{13}C NMR and DEPT spectra revealed the presences of 22 carbon signals in **1**, including two ketocarbonyls (δ_C 209.5 and 211.3), one ester carbonyl (δ_C 170.1), one terminal double bond (δ_C 110.8 and 141.5), four methyls, five sp^3 methylenes, five sp^3 methines (two oxygenated at δ_C 74.4 and 82.6) and three quaternary carbons. The above-mentioned spectroscopic data indicated that compound **1** was a tetracyclic structure, and closely related to atisane diterpenoids previously isolated from this plant [3].

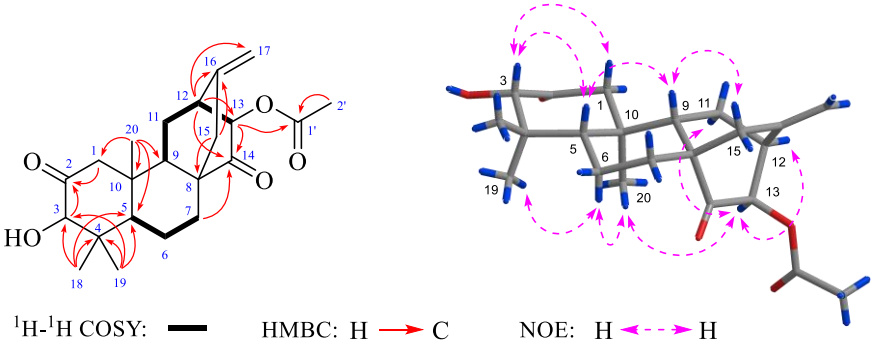

Figure 2. ^{1}H–^{1}H COSY, HMBC, and NOESY correlations of **1**

The ^{1}H–^{1}H COSY correlations (Figure 2) of H-5/H_2-6/H_2-7 and H-9/H_2-11/H-12/H-13, together with HMBC cross-peaks from H_2-1 and H-3 to C-2; from Me-18 and Me-19 to C-3, C-4 and C-5; from Me-20 to C-1, C-5, C-9 and C-10; from H_2-7 and H-13 to C-14; from H-12 to C-16 and C-17; from H_2-15 to C-8 and C-16 established a basic skeleton of the atisane diterpenoid with a typical 6/6/6/6-ring system. The acetyl group was linked to OH-13 by HMBC correlation from H-13 to the acetyl carbonyl (δ_C 170.1). The planar structure of **1** was thus determined. The relative configuration of **1** was established by analyzing its NOE correlations. The NOE correlations of H-3/H-5; H-5/H-9; H-

9/H-15a indicated that they were located on the same face, and H-3, H-5, H-9 and C-8–C-15 bond were randomly assigned as β orientations. Subsequently, the NOE correlations of Me-20/H-13 and H-13/H-12 revealed that they were α-oriented.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for compound **1** (δ in ppm, J in Hz) in $CDCl_3$

No.	δ_H	δ_C	no.	δ_H	δ_C
1α	2.38, d (13.6)	50.6	11β	1.96, m	
1β	2.11, d (13.6)		12	2.81, br dd (3.2, 2.9)	42.8
2		209.5	13	4.95, d (2.9)	74.4
3	3.88, s	82.6	14		211.3
4		45.4	15a	2.36, d (15.0)	42.8
5	1.49, m[a]	53.6	15b	2.31, d (15.0)	
6α	1.50, m[a]	18.8	16		141.5
6β	1.66, m		17a	4.92, s	110.8
7α	2.40, m	30.7	17b	4.81, s	
7β	0.99, m		18	1.17, s	29.4
8		48.0	19	0.64, s	16.4
9	1.91, m	51.4	20	0.68, s	14.5
10		44.1	1′		170.1
11α	1.76, ddd (12.4, 4.6, 3.2)	25.1	2′	2.07, s	20.7

[a] Overlapped signals.

The absolute configuration of **1** was assigned by comparing the experimental data with the calculated ECD results (Figure 3). The calculated ECD curve of **1a** (3*S*,5*S*,8*S*,9*S*,10*R*,12*S*,13*R*-**1**) was in agreement with the measured ECD data of **1**, allowing the determination of the absolute configuration of **1** to be 3*S*, 5*S*, 8*S*, 9*S*, 10*R*, 12*S*, and 13*R*.

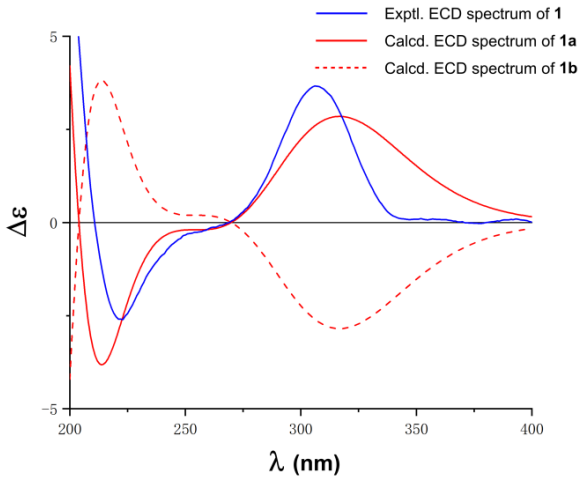

Figure 3. Experimental and calculated ECD spectra of compounds **1**, **1a** (3*S*,5*S*,8*S*,9*S*,10*R*,12*S*,13*R*), and **1b** (3*R*,5*R*,8*R*,9*R*,10*S*,12*R*,13*S*)

The isolated known diterpenoids were identified as *ent*-3*S*-hydroxy-atis-16(17)-en-1,14-dione (**2**) [6], *ent*-(3α,5β,8α,9β,10α,12α)-3-hydroxyatis-16-en-14-one (**3**) [7], *ent*-atis-16-ene-3,14-dione (**4**) [8], *ent*-(13*R*,14*R*)-13,14-dihydroxyatis-16-en-3-one (**5**) [9], antiquorine A (**6**) [10], by comparison of their NMR data with the reported data.

All compounds were tested for their cytotoxicities against HCT-15 cancer cells using the MTT method [11], and only compounds **1** (IC_{50} = 62.98 ± 6.27 μM) and **5** (IC_{50} = 44.35 ± 3.66 μM) showed weak cytotoxicities. Doxorubicin was used as a positive control (IC_{50} = 0.68 ± 0.22 μM).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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