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Ent-Kaurane Diterpenoids from *Euphorbia hirta*

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Abstract: A new *ent*-kaurane diterpenoid was isolated from the ethanol extract of *Euphorbia hirta*, and elucidated as 2*β*,16*α*,19-trihydroxy-*ent*-kaurane (**1**), along with two known *ent*-kauranes, 2*β*,16*α*-dihydroxy-*ent*-kaurane (**2**) and 16*α*,19-dihydroxy-*ent*-kaurane (**3**). Their structures were elucidated on the basis of spectroscopic analysis including 1D and 2D NMR techniques and HRESIMS evidence.

Keywords: Euphorbiaceae; *Euphorbia hirta*;.*ent*-kaurane; diterpenoid.

1. Introduction

Euphorbia is the largest genus in the family of Euphorbiaceae, comprising about 2000 species. More than 80 of them are distributed in China [1]. Many secondary metabolites with specific types of diterpene skeletons in this genus have been found to possess a number of interesting biological activities [2-4]. *Euphorbia hirta* Linn, mainly distributed in the southern and southwestern district of China [5], has been used traditionally for the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis), conjunctivitis, and respiratory diseases (asthma, bronchitis, hay fever) [6,7]. Previous phytochemical studies have reported six diterpenoids of abietane, ingenane and tigliane-types from the species [8,9]. On the course of our search for diterpenoids from *Euphorbia hirta*, a new *ent*-kaurane diterpenoid, 2*β*,16*α*,19-trihydroxy-*ent*-kaurane (**1**), as well as two known *ent*-kauranes (**2** and **3)** were obtained. Among them, compounds **2** and **3** were the first examples as natural products, and NMR data for **2** have not been reported so far. In this paper, we present the isolation and structural elucidation of the new compound (**1**) and the NMR data of **2**.

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2. Materials and Methods

2.1. General procedures

Optical rotations were measured using a Perkin-Elmer model 241 polarimeter. UV spectra were carried out on a Shimadzu UV-2401A spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. 1D and 2D NMR spectra were measured on a Bruker DRX-500 instrument with TMS as internal standard. Mass spectra were obtained on a VG Auto Spec-3000 spectrometer or on a Finnigan MAT 90 instrument. Column chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Inc.), the Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd.), and MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Corp.). Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} on glass plates (Qingdao Marine Chemical Inc.) using various solvent systems.

2.2 Plant Material

The material of plant (*Euphorbia hirta*) was collected in the Xishuang Banna prefecture, Yunnan Province, People's Republic of China, in September 2009. A voucher specimen (YTCM 20090909) was deposited at the Yunnan Traditional Chinese Medical College, and was identified by Prof. Yao-wen Yang.

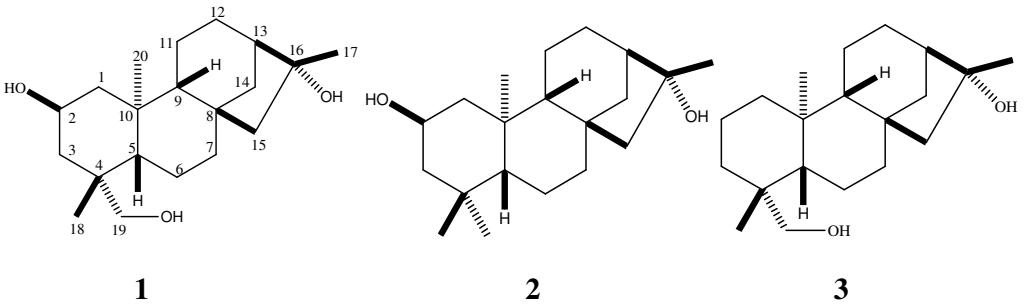
2.3 Extraction and Isolation

Dry herbs of *Euphorbia hirta* (4.0 kg) were extracted with ethanol (3 × 25 L) at room temperature overnight. The extract was partitioned between H_2O and EtOAc, and an EtOAc layer (58 g) was chromatographed on MCI-gel CHP 20P (eluted with 90% CH_3OH–H_2O, then 100% CH_3OH). The 90% CH_3OH fraction (32 g) was repeatedly chromatographed over silica gel eluted in a step gradient manner with petroleum ether–acetone (1:0 to 0:1) to afford fractions A–F. Separation of fraction A (4 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (99:1→1:1), yielded fractions A1–A4. Compounds **2** (2 mg) and **3** (3 mg) were obtained from fraction A1 by repeated chromatography over silica gel with petroleum ether–EtOAc (49:1). Fraction B (5 g) was separated by silica gel column chromatography, eluted with petroleum ether-EtOAc (49:1→1:0), and yielded fractions B1–B4. Fraction B3 afforded compound **1** (2 mg) by chromatography over silica gel with petroleum ether–EtOAc (9:1) and the Sephadex LH-20 column chromatography (100% MeOH).

3. Results and Discussion

Compound **1** was obtained as white powder with $[\alpha]_D^{16.1}$ -60.79 (*c*= 0.16, MeOH), and its molecular formula was determined to be $C_{20}H_{34}O_3$ by HRESIMS data (345.2413 $[M + Na]^+$, calcd 345.2405). IR absorptions at 3386 cm^{-1} indicated the presence of hydroxyl group and UV absorption at 202 nm revealed the absence of unsaturated moiety. The ^{13}C NMR spectrum of **1** (Table **1**) displayed 20 carbon signals corresponding to three methyls, nine methylenes, four methines and four quaternary carbons, which was assigned to be a diterpenoid skeleton. The diagnostic signals of three methines [δ_C 57.6 (C-5), 58.5 (C-9) and 49.0 (C-13)] and three quaternary carbons [δ_C 41.5 (C-4), 46.3 (C-8) and 41.9 (C-10)] indicated *ent*-kaurene skeleton of the diterpenoid. The COSY spectrum showed the presence of three proton sequences of -CH_2 (1)-CH (2)-CH_2 (3)-, -CH (5)-CH_2 (6)-CH_2 (7)- and -CH (9)-CH_2 (11)-CH_2 (12)-CH (13)-CH_2 (14)-, as shown in Figure **2**. An oxygenated methylene (δ_H 3.62 and 3.32, each 1H, d, *J*=11.2 Hz; δ_C 65.7), an oxygenated methine (δ_H 3.87, 1H, m; δ_C 65.0) and an oxygenated quaternary carbon (δ_C 79.7) observed in the ^{13}C NMR spectrum were located at C-19, C-2 and C-16, respectively, which was supported by the HMBC correlations from H-19 to C-4, C-5 and C-18, from H-2 to C-4 and C-10 and from H-12, H-14, H-15 and CH_3-17 to C-16 (Figure **2**). In addition,

the shifts of C-2 were agreement with those of the known compound *ent*-2*α*-hydroxy-8(14),15-pimaradiene [10]. These considerations helped in assigning the planar structure of **1** to be 2,16,19-trihydroxy-*ent*-kaurane.



1 **2** **3**

Figure 1. The structures of compounds **1-3**

The relative configuration of compound **1** was established on the basis of ROESY correlations of H-2 with H-19 and Me-20, H-19 with Me-20, Me-17 with H-12*β*, H-12*β* with H-9*β* as well as 16-OH with H-13*α* as shown in computer-generated 3D drawing (Figure **2**), which suggested the 2-OH and 16-OH possess *β* and α-orientations, respectively. Moreover, the presence of 19-OH was proved by the downfield shift of C-5 (δ_C 57.6) owing to the absence of a *γ*-steric compression effect between 18*β*-OH and H-5. Thus, compound **1** was unambiguously identified as 2*β*,16*α*,19-trihydroxy-*ent*-kaurane.

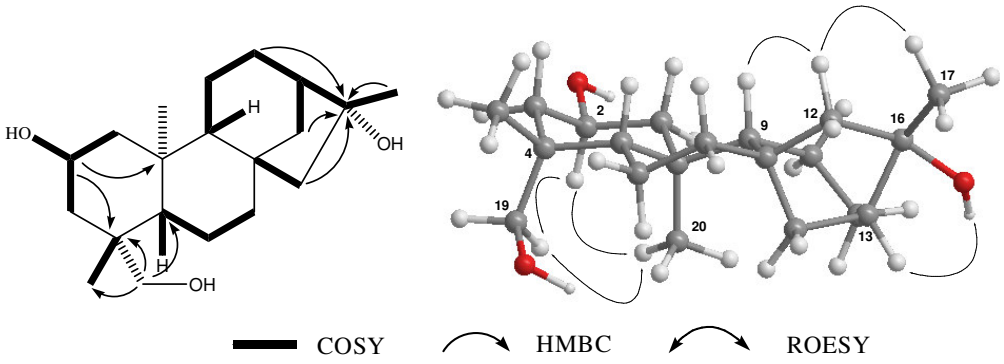


Figure 2. Key HMBC, COSY and ROESY correlations of compound **1**

Compounds **2** and **3** were identified according to the NMR and MS as well as comparison with values from the literature [11].

Table 1. ^{1}H-NMR and ^{13}C-NMR data of compounds **1** and **2** in CD_3OD [a]

	1			**2**	
Position	^{1}H-NMR	^{13}C-NMR	**Position**	^{1}H-NMR	^{13}C-NMR
1α	2.17, m	50.5 (CH_2)	1α	2.16, br d, *J*=11.0 Hz	52.0 (CH_2)
1β	0.68, t, *J*=11.2 Hz		1β	0.65, t, *J*=11.0 Hz	
2α	3.87, m	65.0 (CH)	2α	3.86, m	65.5 (CH)
3α	2.13, m	46.3 (CH_2)	3α	2.14, br d, *J*=11.4 Hz	50.4 (CH_2)
3β	0.82, t, *J*=11.0 Hz		3β	1.83, br s	
4	-	41.5 (C)	4	-	35.7 (C)
5β	0.93, d, *J*=10.8 Hz	57.6 (CH)	5β	0.80, d, *J*=11.8 Hz	57.1 (CH)
6	1.68, m	21.3 (CH_2)	6	1.46, m	21.2 (CH_2)
7α	1.64, overlap	43.5 (CH_2)	7α	1.62, overlap	43.1 (CH_2)
7β	1.45, m		7β	1.47, m	
8	-	46.3 (C)	8	-	46.2 (C)
9β	1.05, d, *J*=4.8 Hz	58.5 (CH)	9β	1.05, d, *J*=4.9 Hz	58.3 (CH)
10	-	41.9 (C)	10	-	42.1 (C)
11	1.64, overlap	19.3 (CH_2)	11	1.60, overlap	19.2 (CH_2)
12	1.63, overlap	27.7 (CH_2)	12	1.59, overlap	27.9 (CH_2)
13α	1.83, br s	49.0 (CH)	13α	3.30, t, *J*=1.4 Hz	49.0 (CH)
14α	1.87, d, *J*=11.6 Hz	38.4 (CH_2)	14α	1.89, d, *J*=11.6 Hz	38.6 (CH_2)
14β	1.60, overlap		14β	1.58, overlap	
15	1.54, s	58.5 (CH_2)	15	1.53, s	58.7 (CH_2)
16	-	79.7 (C)	16	-	79.8 (C)
17	1.33, s	24.4 (CH_3)	17	1.36, s	24.5 (CH_3)
18	0.99, s	28.1 (CH_3)	18	0.86, s	34.2 (CH_3)
19 a	3.62, d, *J*=11.2 Hz	65.7 (CH_2)	19	0.92, s	22.8 (CH_3)
19 b	3.32, d, *J*=11.2 Hz		20	1.09, s	19.4 (CH_3)
20	1.08, s	19.9 (CH_3)			

[a]The assignments are based on DEPT, HSQC and HMBC experiments.

Acknowledgments

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Supporting Information:

The following Supporting Information is available for this article: http://www.acgpubs.org/RNP

References

[1] J. S. Ma and Y. C.Tseng (1997). Euphorbiaceae. Editorial Committee of Flora Reipublicae Popularis Sinicae. Flora of China, Science Press, Beijing. 26.
[2] A. R. Lal, R. C. Cambie, P. S. Rutledge and P. D. Woodgate (1990). *Ent*-atisane diterpenes from *Euphorbia fidjiana*, *Phytochemistry* **29**, 1925-1935.
[3] V. Ravikanth, R. V. L. Niranjan, R. T. Prabhakar, P. V. Diwan, S. Ramakrishna and Y. Venkateswarlu (2002). Macrocyclic diterpenes from *Euphorbia nivulia, Phytochemistry* **59**, 331-335.
[4] V. U. Ahmad, H. Hussain, I. A. Bukhari, J. Hussain, A. R. Jassbi and A. Dar (2005). Antinociceptive diterpene from *Euphorbia decipiens, Fitoterapia* **76**, 230-232.
[5] Academia Sinica (1979). Botany of China, Science Publishing House, Beijing, **44**, 42.
[6] K. S. Mhaskar, E. Blatter and J. F. Caius (2000). *Illustrated Ind. Med. Plant.* Satguru Publications, New Delhi, **9**, 3031.

[7] S. Y. L. Daphne, H. M. Er and Y. S. Chen (2009). Mutagenic and antimutagenic activities of aqueous and methanol extracts of *Euphorbia hirta, J. Ethnopharmacol.* **126**, 406-414.
[8] R. K. Baslas and R. Agarwal (1980). Isolation and characterization of different constituents of *Euphorbia hirta* Linn, *Curr. Science* **49**, 311-312.
[9] A. Hazimi, H. Mohammad, A. Milha and A. Sarra (2008). Jolkinolide diterpenoids and other constituents from *Euphorbia hirta, J. Saudi Chem. Soc*. **12**, 87-93.
[10] G. Topcu, A.C. Goren, T. Kiliç, Y. K. Yildiz, and G. Tümen (2002). Diterpenes from *Sideritis trojana, Nat. Prod. Lett.* **16**, 33-37.
[11] R. H. James, B. H. Peter and A. T. Jacqueline (1995). Biotransformation of *ent*-16*β*,19-Dihydroxykaurane by *Cephalosporium Aphidicola, Phytochemistry* **40**, 797-800.