SHORT REPORT

ACG publications

Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

A New Ingenane Diterpenoid from *Euphorbia jolkinii*

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(Received January 20, 2023; Revised March 07, 2023; Accepted March 09, 2023)

Abstract: A new ingenane diterpenoid, named ingenol-20-laurate (**1**), and two known analogues (ingenol-20-palmitate (**2**), ingenol-20-pentadecanoate (**3**)) and an *ent*-abietane diterpenoid (euphopilolide, **4**) were isolated from the whole plants of *Euphorbia jolkinii*. The structure of compound **1** was elucidated by HRESIMS and 2D NMR methods. The cytotoxicities of compound **1** against SMMC-7721, MDA-MB-231, and SW480 cell lines were 27.22±0.58, 17.69±0.29 and 16.19±0.77 μM, respectively.

Keywords: *Euphorbia jolkinii***;** diterpenoid; cytotoxicity.

1. Plant Source

The whole plants of *Euphorbia jolkinii* were collected in Kunming, Yunnan Province, People's Republic of China in October, 2021, the plants were identified by Dr. Yong Xiong from the School of Ethnic Medicine, Yunnan Minzu University, and its voucher specimen was deposited at Yunnan Minzu University (EJ-202110).

2. Previous Studies

E. jolkinii (or *E. nematocypha*) is distributed in Taiwan, Yunnan, and Sichuan provinces of China, it is a perennial herb for treating ascites, skin itching, and scabies [1]. Literature reported triterpenoids, ellagic and gallic acids, phenylpropanoids, pyranocoumarins [1-4], and diterpenes were isolated from *E. jolkinii*, and the diterpene skeletons from this plant including myrsinol, *ent*-abietane, tigliane, abietane, casbane, lathyrane, ingenane, *ent*-kaurane, and jolkinolide [5-8].

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.384.2301.2685
Available online: March 22, 2023

3. Present Study

The whole plants of *E. jolkinii* (1.7 kg) were air-dried, and then extracted with alcohol three times for 5 h (50 ℃). After the evaporation of the alcohol, the yielded crude extract (520 g) was extracted with CH_2Cl_2 and concentrated (111 g). The residue was separated by an SGC (silica gel column, PE (petroleum ether): EtOAc = 10:0 - 0:10) to afford nine fractions (A-I). Fraction D (40 g) was separated by an SGC (PE: EtOAc = 10:0 - 0:10), and seven fractions (D1-D7) were obtained. D3 (1.4 g) was purified by a preparative HPLC to afford compound **1** (CH_3OH: H_2O = 91: 9, 7 mL/min, t_R = 9.8 min, 17.3 mg), **2** (CH_3OH: H_2O = 93: 7, 6.0 mL/min, t_R = 23.0 min, 188.6 mg) and **3** (CH_3OH: H_2O = 93: 7, 6.0 mL/min, t_R = 11.3 min, 50.1 mg). D4 (1.1 g) was purified by a preparative HPLC to afford **4** (CH_3OH: H_2O = 92.5: 7.5, 6 mL/min, t_R = 3.6 min, 1.8 mg).

*Ingenol-20-laurate (**1**)*: a colorless oil, $[\alpha]_D^{20}$ +7.27 (*c* 0.30, MeOH); for ^{1}H NMR and ^{13}C NMR, see Table 1. ESIMS: *m/z* 553[M+Na]$^+$; HREIMS: *m/z* 553.3505 (calcd. for 553.3505 [M+Na]$^+$).

Cytotoxicity Assay: compound **1** (ingenol-20-laurate) was tested the cytotoxicity by MTS methods [9], and the activities against SMMC-7721 (hepatoma), MDA-MB-231 (breast cancer), and SW480 (colon cancer) cells were 27.22±0.58, 17.69±0.29, and 16.19±0.77 μM in IC_{50}, respectively (Table 2). (See S1 in supporting information for the detail of procedure).

*Alkaline hydrolysis of **1** and GC-MS analysis*: compound **1** (3.0 mg) was dissolved in methyl alcohol (0.006 mmol), and then added K_2CO_3, stirred at 20 ℃ for 2 h. After concentrating, the residue was dissolved in EtOAc and analyzed by GC-MS [10]. The dodecanoic acid, methyl ester was carried out for **1**, giving a peak at 14.73 min and *m/z* 214.

The whole plants of *E. jolkinii* were dried, extracted, and then separated by SGC and preparative HPLC to give compound **1-4** (Figure 1). Compound **1** (ingenol-20-laurate) was elucidated by HRMS and NMR as a new compound, while the known compounds, ingenol-20-palmitate (**2**) [10], ingenol-20-pentadecanoate (**3**) [10] and euphopilolide (**4**) [11] were confirmed by the comparison of the NMR and ESIMS data with literature.

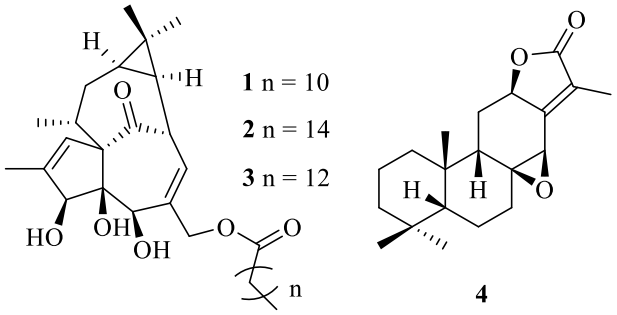

Figure 1. The chemical structures of compounds **1-4**

Compound **1**, a colorless oil, the molecular formula of this compound was speculated as $C_{32}H_{50}O_6$ by HRESIMS *m/z* 553.3505 (calculated for 553.3505, [M+Na]$^+$) and NMR spectra, the degrees of unsaturation was eight. In the ^{1}H NMR spectrum, two alkene protons [δ_H 5.92 (1H, d, *J* = 1.5 Hz, H-1), 6.07 (1H, d, *J* = 3.7 Hz, H-7)] and two oxidized methine protons [δ_H 4.42 (1H, d, *J* = 5.5 Hz, H-3), 3.65 (1H, d, *J* = 10.6 Hz, H-5)], five groups of methyl protons [δ_H 1.06 (3H, s, C$\underline{H}_3$-16), 1.11 (3H, s, C$\underline{H}_3$-17), 0.88 (3H, t, *J* = 6.8 Hz, C$\underline{H}_3$-12′), 0.97 (3H, d, *J* = 6.9 Hz, C$\underline{H}_3$-18), 1.84 (3H, d, *J* = 1.1 Hz, C$\underline{H}_3$-19)], one pair of oxidized methylene protons [δ_H 4.53 (1H, d, *J* = 12.9 Hz, H_b-20), 4.70 (1H, d, *J* = 12.9 Hz, H_a-20)], one pair of methylene protons [δ_H 2.25 (1H, m, H_a-12), 1.74 (1H, m, H_b-12)], and a group of overlapped methylene protons [δ_H 1.26 (16H, m, C$\underline{H}_2$-4′–C$\underline{H}_2$-11′)] were revealed. The ^{13}C NMR spectrum showed the signals of five methyls (δ_C 28.5, 15.5, 17.4, 15.4, 14.1), twelve methylene (δ_C 31.0, 66.3, 34.4, 24.9, 29.1, 29.3, 29.6, 29.6, 29.5, 29.3, 31.9, 22.7), eight

methines (δ_C 129.7, 80.4, 73.8, 127.9, 44.0, 39.7, 23.2, 23.0) and seven quaternary carbons (δ_C 138.9, 84.3, 136.8, 207.0, 72.6, 23.9, 174.3). The information above revealed four degrees of unsaturation, the left four of them revealed that compound **1** was a tetracyclic compound. Analyzing the ^{1}H NMR and ^{13}C NMR spectrum revealed a long aliphatic chain with an ester carbonyl, ten methylenes, and a methyl, and the left twenty carbons implied that compound **1** was a diterpenoid. Furthermore, the comparison of the ^{13}C NMR data of **1** with the literature [10] revealed **1** was an ingenane-type diterpenoid, and it was similar to ingenol-20-pentadecanoate [10], the only difference was there were twelve carbons in the aliphatic chain of **1**.

Table 1. The NMR data of ingenol-20-laurate (**1**) ($CDCl_3$, *J* in Hz)

No	δ_C (100 MHz)	δ_H (400 MHz)	No	δ_C (100 MHz)	δ_H (400 MHz)
1	129.7	5.92, d (1.5)	17	15.5	1.11, s
2	138.9		18	17.4	0.97, d (6.9)
3	80.4	4.42, d (5.5)	19	15.4	1.84, d (1.1)
4	84.3		20	66.3	4.70, d (12.9)
5	73.8	3.65, d (10.6)			4.53, d (12.9)
6	136.8		1′	174.3	
7	127.9	6.07, d (3.7)	2′	34.4	2.30, t (6.5)
8	44.0	4.07, dd (3.8, 11.7)	3′	24.9	1.59, m
9	207.0		4′	29.1	1.26, m
10	72.6		5′	29.3	1.26, m
11	39.7	2.32, m	6′	29.6	1.26, m
12	31.0	2.25, m	7′	29.6	1.26, m
		1.74, brs	8′	29.5	1.26, m
13	23.2	0.70, dd (8.5, 14.9)	9′	29.3	1.26, m
14	23.0	0.94, m	10′	31.9	1.26, m
15	23.9		11′	22.7	1.26, m
16	28.5	1.06, s	12′	14.1	0.88, t (6.8)

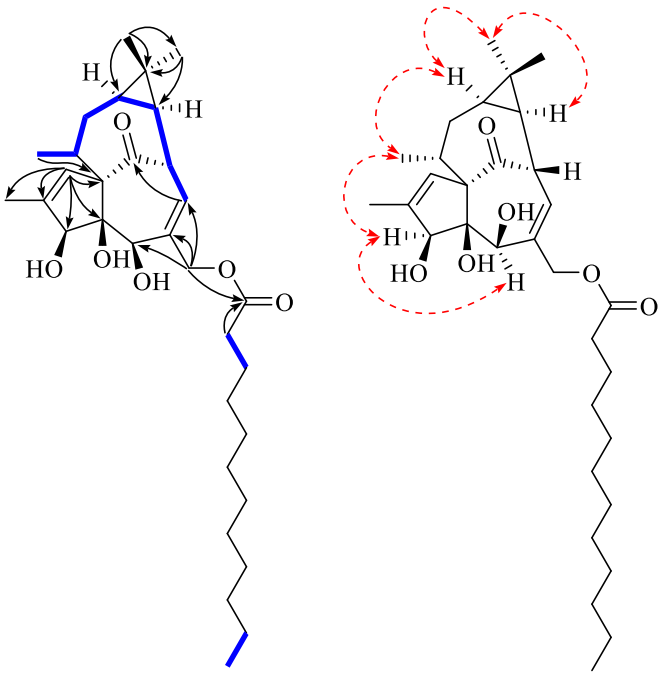

Figure 2. the HMBC (→), ^{1}H-^{1}H COSY (▬) and ROESY (↔) correlations of ingenol-20-laurate (**1**)

The structure of compound **1** was confirmed by analysis of HSQC, HMBC, and ^{1}H-^{1}H COSY spectra. The cross peaks of the ^{1}H-^{1}H COSY of H-7/H-8/H-14/H-13/Hb-12/H-11/C$\underline{H_3}$-18, and the cross peaks of HMBC from C$\underline{H_3}$-16 to C-13, C-14, C-15 and C-17, H-1 to C-2, C-3, C-4, C-10, C-11 and C-19, H-7 and H-11 to C-9, H_a-20 and H_b-20 to C-5, C-6 and C-7 confirmed the ingenane skeleton. Besides, the cross peaks of ^{1}H-^{1}H COSY of C$\underline{H_2}$-2′/C$\underline{H_2}$-3′ and C$\underline{H_2}$-11′/C$\underline{H_3}$-12′ with the HMBC cross peaks from H_a-20, H_b-20, and C$\underline{H_2}$-2′ to C-1′ revealed the long aliphatic chain at C-20.

The relative configuration of **1** was assigned by the ROESY and by comparison of the NMR data with the literature. The cross peaks of ROESY of H-5/H-3/C$\underline{H_3}$-18/H-13/C$\underline{H_3}$-17/H-14, and the ^{1}H NMR signals of these protons were close to ingenol-20-pentadecanoate[9], as a result, these protons were assigned as α-configurations. The configurations of H-8 and C$\underline{H_3}$-16 were confirmed as β-configurations by the comparison of their ^{1}H NMR signals with ingenol-20-pentadecanoate. Thus, the structure of compound **1** was assigned, it was named ingenol-20-laurate, as illustrated in Figure 1.

Table 2. cytotoxicity assay of compound **1**

Compound	SMMC-7721	MDA-MB-231	SW480
1	27.22±0.58	17.69±0.29	16.19±0.77
DDP	8.098±0.882	16.90±1.19	25.06±1.26
Taxol	0.121±0.004	<0.008	<0.008

Acknowledgments

This study was financed by the Scientific Research Foundation of Yunnan Provincial Department of Education (2023J0596), Open Funding of Key Laboratory of State Forestry and Grassland Administration on Highly-efficient Utilization of Forestry Biomass Resources in Southwest China (2021-KF07), Yunnan Applied Basic Research Projects for Excellent Young Scholars (202001AW070002) and the Candidates of the Young and Middle Aged Academic Leaders of Yunnan Province (2019HB016).

Supporting Information

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

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