SHORT REPORT

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Cytotoxic Lathyrane Diterpenoids from the Roots of *Euphorbia fischeriana*

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Abstract: Two new lathyrane diterpenoids, euphofischers A and B (**1** and **2**), along with three known analogues (**3**–**5**), were isolated from the roots of *Euphorbia fischeriana*. Their structures were elucidated by spectroscopic analysis. Euphofischer A (**1**) represents rare example of a lathyrane diterpenoid featuring a 15-*p*-coumaroyl moiety. All compounds were screened for their cytotoxicity against three cancer cell lines (C4-2B, C42B/ENZR, and MDA-MB-231), and compound **1** exhibited significant cytotoxicity against C4-2B cell line, with an IC_{50} value of 11.3 μM.

Keywords: *Euphorbia fischeriana*; Euphorbiaceae; lathyrane; diterpenoid.

1. Plant Source

In the current phytochemical investigation regarding traditional Chinese medicine (TCM), two new lathyrane diterpenoids and three known analogues were isolated from the roots of *Euphorbia fischeriana* Steud. (Euphorbiaceae) (Plant materials see supporting information). We report herein the isolation, structure elucidation, and cytotoxicities of these compounds (Figure 1).

2. Previous Studies

Euphorbia fischeriana, a perennial herb widely distributed in northern provinces of China, is known as "Lang Du" in traditional Chinese medicine (TCM) for its treatment of tuberculosis, edema, and cancer [1,2]. Previous phytochemical studies on this plant have demonstrated that lathyrane, tigliane, *ent*-atisane, *ent*-abietane diterpenoids and diverse terpenoids are the main constituents of *E. fischeriana*, and some of them possessed significant biological properties, such as antituberculosis, cytotoxic, antiinflammatory and anti-HIV activities [3–8].

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3. Present Study

The EtOAc extract of the roots of *E. fischeriana* was separated with a series of column chromatographic method to afford compounds **1**–**5** (detailed separation process see supporting information). Their structures were identified as shown in Figure 1.

*Euphofischer A (**1**):* $[\alpha]^{20}_{D}$ −188.2 (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 273 (4.04) nm; IR (KBr) ν_{max} 3391, 2930, 1703, 1603, and 1153 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS m/z 481.2589 $[M + H]^+$ (calcd. for $C_{29}H_{37}O_6^+$, 481.2585) and 479.2430 $[M - H]^-$ (calcd. for $C_{29}H_{35}O_6^-$, 479.2439).
*Euphofischer B (**2**):* $[\alpha]^{20}_{D}$ −70.7 (*c* 0.90, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 277 (4.40) nm; IR (KBr) ν_{max} 3481, 2933, 1717, 1633, 1235, and 1153 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS m/z 545.2517 $[M + Na]^+$ (calcd. for $C_{31}H_{38}O_7Na^+$, 545.2510).

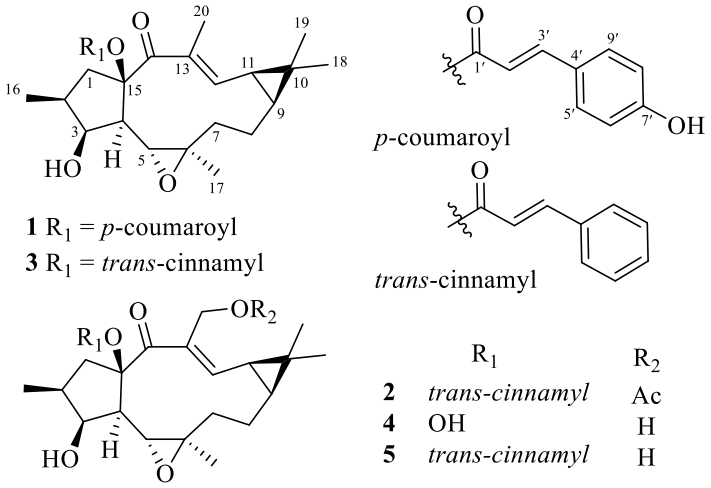

Figure 1. Structures of compounds **1**–**5**

Compound **1** was isolated as a white powder. The pseudomolecular-ion peaks in the HRESIMS at *m/z* 481.2589 for $[M + H]^+$ (calcd. for $C_{29}H_{37}O_6^+$ 481.2585) and at *m/z* 479.2430 for $[M - H]^-$ (calcd. for $C_{29}H_{35}O_6^-$ 479.2439) allowed the molecular formula $C_{29}H_{36}O_6$ to be assigned to **1**. The IR spectrum absorption bands at 3391 cm^{-1} and 1703 cm^{-1} suggested the presences of hydroxyl and carbonyl groups, respectively. The ^1H NMR data (Table 1) showed the signals for five methyl groups (δ_H 0.81, 1.09, 1.10, 1.18, and 1.86), three olefinic protons (δ_H 6.21, 6.97, and 7.56), two oxymethine protons (δ_H 3.67 and 4.15), a disubstituted phenyl (δ_H 6.82 and 7.30), and a series of aliphatic multiplets. The ^{13}C NMR spectrum, associated with the DEPT and HSQC experiments (Table 1), classified 29 carbon resonances for a conjugated ketocarbonyl (δ_C 195.2), a *p*-coumaroyl group (δ_C 114.5, 115.9 × 2, 126.5, 130.1 × 2, 146.2, 158.5, and 166.0), a trisubstitued double bond (δ_C 134.0 and 144.4), two oxygenated sp^3 tertiary carbons (δ_C 64.5 and 91.7), five methyls, three sp^3 methylenes, six sp^3 methines (two oxymethines at δ_C 58.5 and 78.8), and a quaternary carbon. The above-mentioned NMR data exhibited a high similarity to that of a co-isolated known compound jolkinol B (**3**) [9] (Table S1), except for the replacement of a *trans*-cinnamyl group in **3** by a *p*-coumaroyl group in **1**. This was supported by comparison of the ^1H and ^{13}C NMR data of *p*-coumaroyl moiety in **1** with those of *p*-coumaric acid [10], as well as the HMBC correlations from H-5′/H-9′ and H-6′/H-8′ to an aromatic oxygenated quaternary carbon (δ_C 158.5, C-7′). Detailed analysis of 2D NMR data, especially the ^1H–^1H COSY and HMBC correlations, supported the assignment of the planar structure of **1** as shown in Figure 1.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data for **1** and **2** (δ in ppm)

No.	**1**		**2**	
	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C
1α	3.53, dd (13.3, 7.6)	45.0	3.53, dd (13.4, 7.4)	44.3
1β	1.73, dd (13.3, 13.3)		1.75, dd (13.4, 13.4)	
2	2.02, m	38.5	2.02, m	38.4
3	4.15, dd (3.7, 3.7)	78.8	4.14, dd (3.6, 3.6)	78.5
4	1.57, dd (9.4, 3.7)	52.1	1.63, m	51.6
5	3.67, d (9.4)	58.5	3.64, d (9.3)	58.1
6		64.5		63.6
7a	1.62, m	38.6	1.63, m	38.5
7b	2.05, m		2.05, m	
8α	2.01, m	23.2	2.10, m	23.3
8β	1.51, m		1.56, m	
9	1.12, m	33.8	1.27, m	35.2
10		26.3		28.0
11	1.47, dd (11.0, 7.8)	29.7	1.67, dd (10.8, 7.8)	29.8
12	6.97, d (11.0)	144.4	7.27, d (10.8)	150.7
13		134.0		132.4
14		195.2		193.1
15		91.7		91.8
16	1.10, d (6.9)	13.2	1.10, d (6.7)	13.1
17	1.18, s	20.0	1.24, s	20.0
18	1.09, s	29.0	1.12, s	29.0
19	0.81, s	16.1	0.87, s	16.2
20	1.86, s	12.4	a 4.92, d (11.5) b 4.98, d (11.5)	58.1
1′		166.0		165.4
2′	6.21, d (15.8)	114.5	6.44, d (16.0)	117.1
3′	7.56, d (15.8)	146.2	7.69, d (16.0)	146.8
4′		126.5		133.8
5′/9′	7.30, d (8.5)	130.1	7.47, d (7.5)	128.2
6′/8′	6.82, d (8.5)	115.9	7.40, m	129.0
7′		158.5	7.40, m	130.8
-OO<u>C</u>CH$_3$				170.9
-OOC<u>C</u>H$_3$			1.98, s	20.9

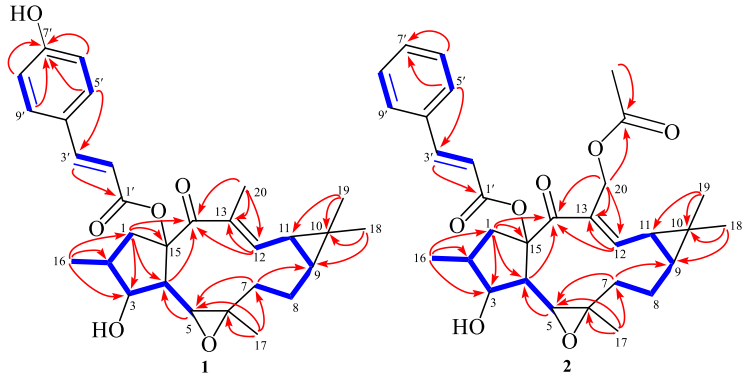

Figure 2. ^{1}H–^{1}H COSY (▬) and key HMBC (→) correlations of **1** and **2**

Interpretations of the NOE correlations and $^{1}H–^{1}H$ coupling constants (Figure 3) allowed the assignments of the relative configuration of compound **1**. Firstly, the strong NOE correlations of H-4/H_3-17 and H-5/H-8β indicated that H-5 was in a *trans*-positon of CH_3-17 on the epoxy ring, which was further confirmed by comparison of the NMR data of C-5 and C-6 in **1** with those of known analogue jolkinol B (**3**) (Table S1). Thus, H-4 and CH_3-17 were arbitrarily designated as α-orientations, while H-5 was β-oriented. Subsequently, the NOE correlations from H-3 to H-2 and H-4 and from H-2 to H-4, together with small coupling constant of H-3 (dd, J = 3.7, 3.7 Hz) indicated that H-2 and H-3 were α-oriented [11]. A *cis*-orientation for the β-oriented dimethylcyclopropane moiety was established by the NOE correlations of H-9/H-11 and H-8β/CH_3-19. The NOE correlation of H-11/CH_3-20 determined the *E*-geometry for $\Delta^{12(13)}$ double bond. The *trans*-relationship of H-4 and 15-*p*-coumaroyl was proposed to be the same as that **3** and other analogues based on biosynthetic considerations and its ^{13}C chemical shifts at C-15 (δ_C 91.7) and C-4 (δ_C 52.1) [9, 11−13]. Thus, the structure of **1** was determined and was given a trivial name euphofischer A.

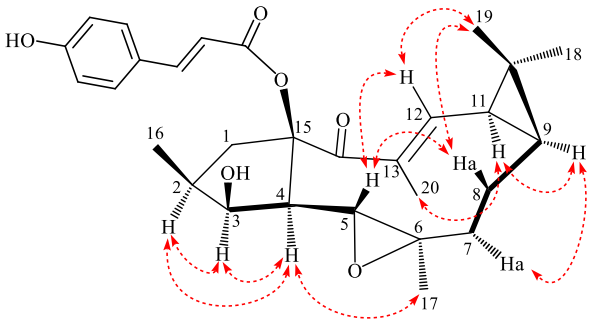

Figure 3. key NOE (↔) correlations of **1**

Compound **2** showed a molecular formula $C_{31}H_{38}O_7$ as determined by its HRESIMS data. Its 1D NMR data resembled that of jolkinol A (**5**) [14] (Table S1), with the major difference being the presence of an additional acetyl group (δ_H 1.98; δ_C 170.9 and 20.9) in **2**, indicating that **2** was an acetylated derivative of **5**. HMBC correlations from H_a-20 and H_b-20 to the additional carbonyl carbon assigned that the acetyl group was located at OH-20. This was further supported by the severely downfield-shifted H_2-20 signals in **2** with respect to that in **5** (δ_H 4.92 and 4.98 in **2**; δ_H 4.30 and 4.49 in **5**). The relative configuration of **2** was assigned to be the same as that of **5** based on their similar 1D NMR data and NOESY correlations. Compound **2** was named euphofischer B.

The known compounds jolkinol B (**3**) [9], ebracteolata C (**4**) [15], and jolkinol A (**5**) [14], were identified by comparison of their NMR data with those in the literature.

All the isolates were investigated the cytotoxicity against three cancer cells [C4-2B (human prostate cancer cells) and C4-2B/ENZR (enzalutamide-resistant C4-2B cell line), and MDA-MB-231 (human breast cancer cells)] by using the reported method [5,16]. As a result, most of these compounds showed moderate activities against C4-2B and C4-2B/ENZR cell lines with IC_{50} values ranging from 20.9 to 37.3 μM, but showed weak activities against MDA-MB-231 cell line (IC_{50} > 50 μM). Particularly, compound **1** exhibited significant cytotoxicity against C4-2B cell line with an IC_{50} value of 11.3 μM (Table 2).

Table 2. Cytotoxicities of **1**–**5** against three cancer cell lines.

Cell lines	IC_{50} (μM)					
	1	**2**	**3**	**4**	**5**	DOX[a]
C4-2B	11.3 ± 0.66	21.3 ± 2.66	34.3 ± 1.34	25.3 ± 0.86	20.9 ± 0.62	0.23 ± 0.33
C4-2B/ENZR	23.2 ± 1.22	26.8 ± 2.88	37.3 ± 3.66	35.1 ± 2.84	21.9 ± 1.96	0.76 ± 0.23
MDA-MB-231	> 50	> 50	> 50	> 50	> 50	0.92 ± 0.34

[a] Positive control:doxorubicin.

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Supporting Information

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

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