

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 IC₅₀ value of 11.3 μM.

Keywords: *Euphorbia fischeriana*; Euphorbiaceae; lathyrane; diterpenoid. © 2020 ACG Publications. All rights reserved.

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, tiglane, *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): $[\alpha]_D^{20} -188.2$ (*c* 0.30, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 273 (4.04) nm; IR (KBr) ν_{\max} 3391, 2930, 1703, 1603, and 1153 cm⁻¹; ¹H and ¹³C NMR data see Table 1; HRESIMS *m/z* 481.2589 [M + H]⁺ (calcd. for C₂₉H₃₇O₆⁺, 481.2585) and 479.2430 [M – H]⁻ (calcd. for C₂₉H₃₅O₆⁻, 479.2439).

Euphofischer B (2): $[\alpha]_D^{20} -70.7$ (*c* 0.90, CHCl₃); 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 ¹³C NMR data see Table 1; HRESIMS *m/z* 545.2517 [M + Na]⁺ (calcd. for C₃₁H₃₈O₇Na⁺, 545.2510).

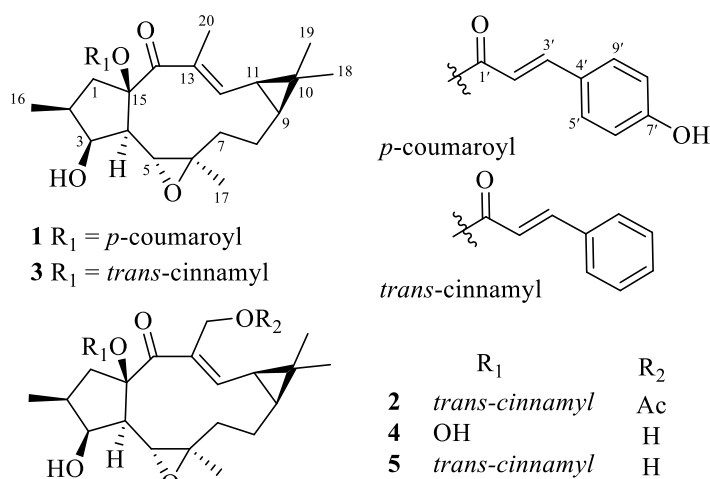
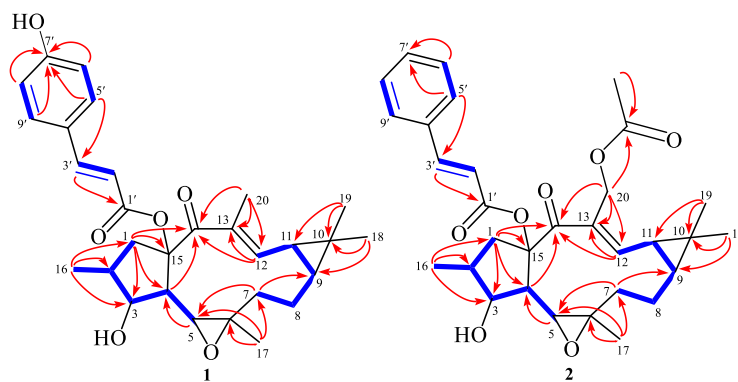


Figure 1. Structures of compounds **1–5**

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 C₂₉H₃₇O₆⁺ 481.2585) and at *m/z* 479.2430 for [M – H]⁻ (calcd. for C₂₉H₃₅O₆⁻ 479.2439) allowed the molecular formula C₂₉H₃₆O₆ 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 ¹³C 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 trisubstituted double bond (δ_{C} 134.0 and 144.4), two oxygenated sp³ tertiary carbons (δ_{C} 64.5 and 91.7), five methyls, three sp³ methylenes, six sp³ 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 ¹³C 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. ^1H 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}
1 α	3.53, dd (13.3, 7.6)	45.0	3.53, dd (13.4, 7.4)	44.3
1 β	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	
8 α	2.01, m	23.2	2.10, m	23.3
8 β	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
-OOC <u>CH</u> ₃				170.9
-OOC <u>C</u> CH ₃			1.98, s	20.9

**Figure 2.** ^1H - ^1H COSY (—) and key HMBC (—) correlations of **1** and **2**

Interpretations of the NOE correlations and ^1H - ^1H coupling constants (Figure 3) allowed the assignments of the relative configuration of compound **1**. Firstly, the strong NOE correlations of H-4/H₃-17 and H-5/H-8 β indicated that H-5 was in a *trans*-position of CH₃-17 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 CH₃-17 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-8 β /CH₃-19. The NOE correlation of H-11/CH₃-20 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.

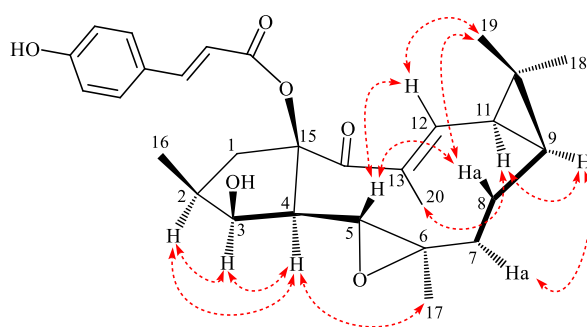


Figure 3. key NOE ($\leftarrow\cdots\rightarrow$) correlations of **1**

Compound **2** showed a molecular formula $\text{C}_{31}\text{H}_{38}\text{O}_7$ 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 H₂-20 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 ($\text{IC}_{50} > 50$ 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)					DOX ^a
	1	2	3	4	5	
C4-2B	11.3 \pm 0.66	21.3 \pm 2.66	34.3 \pm 1.34	25.3 \pm 0.86	20.9 \pm 0.62	0.23 \pm 0.33
C4-2B/ENZR	23.2 \pm 1.22	26.8 \pm 2.88	37.3 \pm 3.66	35.1 \pm 2.84	21.9 \pm 1.96	0.76 \pm 0.23
MDA-MB-231	> 50	> 50	> 50	> 50	> 50	0.92 \pm 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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