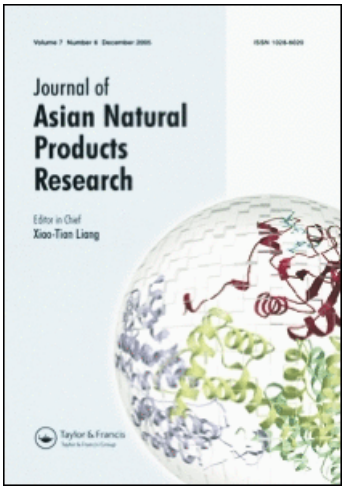

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Six tigliane-type diterpenoids (**1**–**6**) were isolated from the roots of *Euphorbia fischeriana*. Their structures were elucidated by various spectral analyses. Among them, compounds **1** and **3** were new, and compounds **2**, **4**, and **5** were naturally obtained for the first time. All compounds were tested against two human cancer cell lines, MDA-MB-231 and HepG2, and one human immortalized cell line, and only compound **6** showed cytotoxicity for MDA-MB-231 cells with an IC_{50} value of 6.694 μM.

Keywords: Euphorbiaceae; *Euphorbia fischeriana*; tigliane diterpenoids; cytotoxic

1. Introduction

Euphorbia fischeriana Steud (Euphorbiaceae) is a perennial herbaceous plant distributed widely in northeast mainland China. The dried plant roots, named 'lang-du' in traditional Chinese medicine, are used as a remedy for the treatment of edema, ascites, and cancer [1]. The chemical constituents of the roots have been investigated, resulting in the isolation of a variety of diterpenoids [2–8]. Two *ent*-abietane diterpenoids, jolkinolides A and B, the major components of the roots, showed significant antitumor activities against sarcoma 180 and Ehrlich ascites carcinoma in mice [9,10]. Recently, we have found that 17-acetoxyjolkinolide B is a novel NF-κB pathway inhibitor, inducing strongly the apoptosis of tumor cells and becoming a promising drug candidate [11]. In our continuing studies on the title plant's roots, six tigliane-type diterpenoids (**1**–**6**) were isolated. Among them, compounds **1** and **3** were new and compounds **2**, **4**, and **5** were naturally obtained for the first time (Figure 1). All compounds were tested against two human cancer cell lines, MDA-MB-231 and HepG2, and one human immortalized cell line.

2. Results and discussion

Compound **1**, a colorless gum, exhibited a molecular formula as $C_{20}H_{26}O_5$ by HR-EI-MS at *m/z* 346.1760. The IR spectrum showed strong absorption bands at 3430, 1701, and 1675 cm^{-1} ascribed to hydroxyl, ketone, and conjugated carbonyl groups, respectively. Absorption at 235 nm in the UV spectrum confirmed the presence of α,β-unsaturated carbonyl group. The ^1H NMR spectrum (Table 1) showed signals for four methyls at δ 1.86 (s), 1.78 (s, 6H), 0.98 (d, $J = 6.4$ Hz), two olefinic methines at δ 7.59 (s), 5.46 (d, $J = 6.8$ Hz), and one oxygenated methylene at δ 3.94 (ABq, $J = 12.8$ Hz). The ^{13}C NMR spectrum

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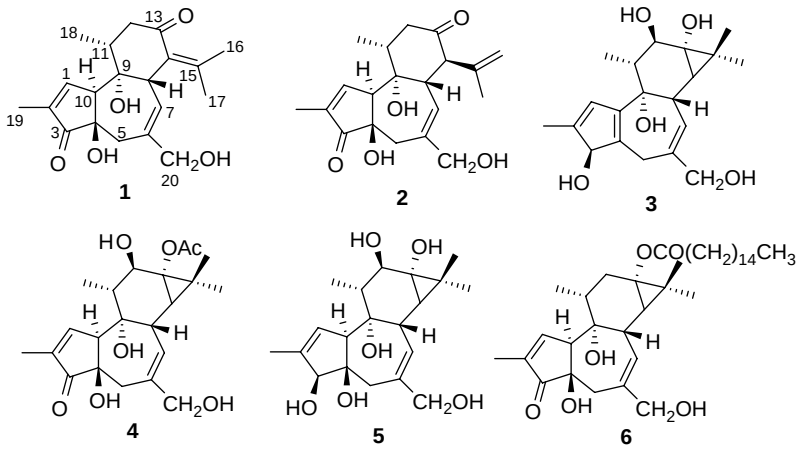

Figure 1. The structures of compounds **1**–**6**.

(Table 2) revealed the signals for four methyl (three olefinic), three methylene (one oxygenated), five methine (two olefinic), and eight quaternary (four olefinic, two carbonyls, two oxygenated) carbons. From above observations and by comparing with previously reported compounds from the title plant, **1** was suggested to be a tigliane diterpenoid. Further studies on its ^{1}H and ^{13}C NMR spectral data revealed that these data of **1** were very similar to those of langduin A, a tigliane diterpenoid from the same plant [3]. The only difference found was that **1** had two quaternary olefinic signals at δ 136.8 (C-14) and 144.5 (C-15) instead of two methines at C-14 and C-15 as in langduin A, suggesting a propylene group at C-14 of **1** instead of an isopropyl group in langduin A. This was supported by HMBC correlations of H-8 with C-14 and C-15; H-16 and H-17 with C-14 as shown in Figure 2. Thus, **1** was deduced as didehydrolangduin A. Furthermore, the HMBC, HMQC, ROESY, and ^{1}H–^{1}H COSY spectra permitted full assignments of ^{1}H and ^{13}C NMR spectral data of **1**.

Compound **2**, a colorless amorphous solid, exhibited the molecular formula

Table 1. ^{1}H NMR spectral data of **1**–**3** in CD_3OD (400 MHz, δ in ppm, J in Hz).

H	1	2	3
1	7.59 (s)	7.61 (s)	6.19 (s)
3			4.50 (s)
5α	2.65 (d, $J = 18.8$)	2.65 (m)	2.38 (d, $J = 19.0$)
5β	2.54 (d, $J = 18.8$)	2.51 (m)	2.28 (d, $J = 19.0$)
7	5.46 (d, $J = 6.8$)	5.44 (d, $J = 6.0$)	5.51 (br s)
8	4.47 (d, $J = 6.8$)	3.65 (dd, $J = 13.2$, 6.0)	2.63 (t, $J = 5.3$)
10	3.34 (s)	3.30 (s)	
11	3.18 (m)	3.17 (m)	2.14 (m)
12	2.36 (dd, $J = 17.2$, 8.0)	2.49 (m)	4.65 (br s)
	2.18 (dd, $J = 17.2$, 8.0)	2.38 (dd, $J = 15.4$, 7.5)	
14		3.46 (d, $J = 13.2$)	1.07 (d, $J = 5.3$)
Me(16) or CH_2(16)	1.78 (s)	4.97 (s), 4.81 (s)	1.87 (s)
Me(17)	1.78 (s)	1.70 (s)	1.20 (s)
Me(18)	0.98 (d, $J = 6.4$)	0.97 (d, $J = 6.8$)	0.89 (d, $J = 6.4$)
Me(19)	1.86 (s)	1.79 (br s)	1.82 (s)
CH_2(20)	3.94 (ABq, $J = 12.8$)	3.95 (br s)	4.15 (ABq, $J = 12.9$)

Table 2. ^{13}C NMR spectral data (100 MHz) of **1**–**3** in CD_3OD (ppm).

C	1	2	3
1	159.9	160.2	126.1
2	142.2	142.8	132.7
3	208.1	210.8	77.5
4	75.3	75.2	137.6
5	38.2	38.7	34.0
6	135.1	136.0	136.2
7	130.9	127.5	128.3
8	50.3	46.2	38.9
9	77.7	77.6	80.7
10	58.9	59.7	132.9
11	39.1	40.3	31.2
12	48.1	48.0	75.6
13	210.5	213.5	75.4
14	136.8	59.2	29.7
15	144.5	143.6	20.7
16	22.8	117.1	25.1
17	23.9	20.5	16.2
18	19.1	18.8	19.0
19	10.8	10.8	20.2
20	68.3	68.8	64.2

$C_{20}H_{26}O_5$ by HR-EI-MS at *m/z* 346.1765. The IR spectrum showed strong absorption bands at 3410, 1705, and 1680 cm^{-1} ascribed to hydroxyl, ketone, and conjugated carbonyl functionalities, respectively. Absorption at 242 nm in UV spectrum confirmed the presence of an α,β-unsaturated carbonyl group. The ^1H NMR spectrum (Table 1) of **2** displayed the signals corresponding to four olefinic protons (δ 7.61, 5.44, 4.97, and 4.81), two olefinic methyls (δ 1.79, 1.70) and one secondary methyl (δ 0.97), and one oxygenated methylene at δ 3.95. The ^{13}C NMR spectrum (Table 2) showed three methyl (two olefinic), four methylene (one oxygenated, one olefinic), six methine (two olefinic), and seven quaternary (two oxygenated) carbons. The above NMR spectral data were similar to those of **1**. The only difference found was that **2** had a double bond between C-15 and C-16 instead of that between C-14 and C-15 in **1**. This was supported by the HMBC correlations of H-8 with C-15; H-14 with C-15 and C-16; and H-16 with C-14, C-15 and C-17 (Figure 2). Literature investigation revealed that the planar structure of **2** was reported in 1967 [12], which was obtained by the treatment of phorbol, a tigliane diterpenoid, with sulfuric acid, but its NMR spectral data and stereostructure have not been determined yet. The α position of H-14 was deduced by the presence of correlation between H-14 (δ 3.46) and H-18 (δ 0.97) and absence of correlation between H-8 (δ 3.65) and H-14 on the basis of ROESY spectrum. Thus, **2** was determined as a new natural product named as langduin F. The unambiguous assignments of its ^1H and ^{13}C NMR spectral data were made by ^1H–^1H COSY, HMQC, HMBC, and ROESY analysis.

Compound **3**, a colorless gum, exhibited a molecular formula of $C_{20}H_{28}O_5$ by HR-EI-MS at *m/z* 348.1926. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed the signals of four methyl (one olefinic, one secondary, two tertiary), two methylene (one oxygenated), seven methine (two olefinic, two oxygenated), and seven quaternary (two oxygenated, two olefinic) carbons, whose structural feature is similar to that of phorbol, a tigliane diterpenoid [13,14]. Further studies revealed that an α,β-unsaturated ketone system in ring A of

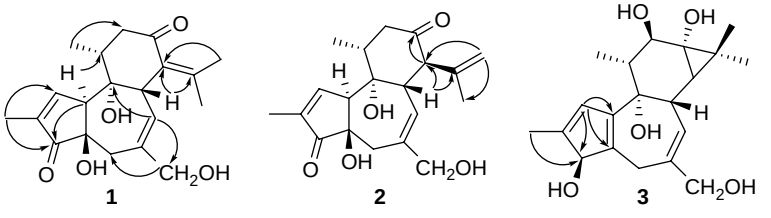

Figure 2. The key HMBC correlations of compounds **1**–**3**.

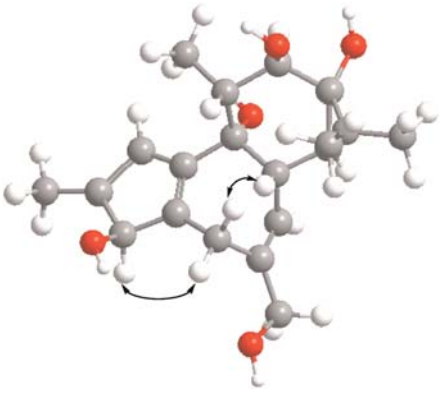

Figure 3. Important ROESY cross-peaks of compound **3**.

phorbol has been changed to an α,α′,β,β′-unsaturated cyclopentanol (δ 126.1, C-1; 132.7, C-2; 77.5, C-3; 137.6, C-4; 132.9, C-10; δ 6.19, s, H-1; 4.50, s, H-3). The HMBC correlations of H-1 and H-19 with C-3; H-1 with C-4 and C-10 confirmed the above deduction (Figure 2). The relative configuration of H-3 was determined as β position on the basis of ROESY correlations of H-3 (δ 4.50) with H-5α (δ 2.38); H-5β (δ 2.28) with H-8 (δ 2.63) (Figure 3). The assignments of all protons and carbons were made unambiguously by $^{1}H-^{1}H$ COSY, HMQC, HMBC, and ROESY evidence. Based on the above evidence, the structure of **3** was determined as 3-hydroxyl-4,10-dehydrophorbol.

Compounds **4**–**6** were identified by various spectral analysis and comparison with literature values. Compound **4** was a degradation product obtained by hydrolysis of phorbol diester [15], and compound **5** was a reduction product of phorbol triacetate with lithium aluminum hydride [16]. Compounds **4** and **5** were naturally obtained for the first time, and known compound **6** was isolated from the title plant [3].

All isolated compounds were evaluated for cytotoxicity against two human cancer cell lines, MDA-MB-231 and HepG2, as well as one human immortalized cell line, HEK293. Only compound **6** (IC_{50} 6.694 μM) was found to be cytotoxic against the MDA-MB-231 cells.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer model 341 polarimeter. UV spectra were recorded by a Shimadzu UV-2550 UV–vis spectrophotometer. IR spectra were recorded using a Nicolet Magna FT-IR spectrophotometer. NMR spectra were run in CD_3OD on a Varian Mercury NMR spectrometer at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. EI and HR-EI-MS were measured using a Finnigan/MAT 95 mass spectrometer. Column chromatographic separations were carried out on silica gel H-60 (Qingdao Marine Chemical Group Corporation, Qingdao, China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and C_{18} reverse-phased SiO_2 (20–45 μm, Fuji Silysia Chemical Ltd, Kasugai, Japan). HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, China) were used for analytical TLC. All solvents used were of chemical grade (Shanghai Chemical Co. Ltd, Shanghai, China).

3.2 Plant material

The roots of *E. fischeriana* were purchased from Shanghai Xuhui TCM Store in October 2006 and identified by Prof. Bing-Yang Ding, School of Life and Environmental Sciences, Wenzhou Normal College. A voucher specimen (No. 200611163) is deposited in the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The powdered whole plant of *E. fischeriana* (5 kg) was percolated with 95% EtOH. After evaporation of the solvent, the crude extract (450 g) was dispersed in H_2O and

then extracted with $CHCl_3$ to afford a residue (110 g), which was subjected to SiO_2 (200–300 mesh), and eluted with petroleum ether–acetone (50:1, 20:1, 10:1, 5:1, 1:1) to yield four subfractions (Fractions A–D). Fraction A (15 g) was chromatographed by repeated SiO_2 CC, eluted with petroleum ether–acetone (30:1, 20:1, 10:1), and RP-18 flash CC, eluted with MeOH–H_2O (60:40) to afford compounds **1** (6 mg), **2** (7 mg), and **6** (10 mg). Fraction B (20 g) was subjected to SiO_2 CC (200–300 mesh), eluted with petroleum ether–acetone (25:1, 15:1, 10:1) to yield three subfractions (Fractions B-1–B-3). Fraction B-1 (1.2 g) was chromatographed by repeated RP-18 flash CC, eluted with MeOH–H_2O (60:40), and Sephadex LH-20 column, eluted with MeOH to afford compounds **3** (9 mg) and **4** (8 mg). Compound **5** (13 mg) was isolated by the same methods.

Compound **1**, colorless gum. $[\alpha]_D^{20}$ +60.1 ($c = 0.105$, MeOH). UV (MeOH): λ_{max} 235 (3.83) nm. IR (KBr): ν_{max} 3430, 1701, 1675 cm^{-1}. ^1H and ^{13}C NMR spectral data (CD_3OD): Tables 1 and 2. HR-EI-MS: *m/z* 346.1760 $[M]^+$ (calcd for $C_{20}H_{26}O_5$, 346.1781).

Compound **2**, colorless amorphous solid. $[\alpha]_D^{20}$ +65.2 ($c = 0.110$, MeOH). UV (MeOH): λ_{max} 242 (3.76) nm. IR (KBr): ν_{max} 3410, 1705, 1680 cm^{-1}. ^1H and ^{13}C NMR spectral data (CD_3OD): Tables 1 and 2. HR-EI-MS: *m/z* 346.1765 $[M]^+$ (calcd for $C_{20}H_{26}O_5$, 346.1781).

Compound **3**, colorless gum. $[\alpha]_D^{20}$ +20.0 ($c = 0.112$, MeOH). UV (MeOH): λ_{max} 230 (3.80) nm. IR (KBr): ν_{max} 3400, 1705, 1670 cm^{-1}. ^1H and ^{13}C NMR spectral data (CD_3OD): Tables 1 and 2. HR-EI-MS: *m/z* 348.1926 $[M]^+$ (calcd for $C_{20}H_{28}O_5$, 348.1938).

3.4 Cytotoxicity assay

Cytotoxicity against the HepG2, MDA-MB-231, and HEK293 cells was evaluated by using the MTT method. Briefly, about 5000 cells/well were seeded into 96-well plates. Twenty four hours later, cells were treated with vehicle control (DMSO) or increasing concentrations of the tested compounds for 72 h. Twenty microliters of 5 mg/ml of MTT solution was then added to each well. After 3–4 h of incubation at 37°C, equal volume of the extraction buffer (10% SDS, 5% isobutanol, and 0.01 mol/L hydrochloric acid) was added to each well. The cells were incubated overnight at 37°C, and the absorbance was then measured at 570 nm using a 96-well multiscanner (Molecular Devices, Sunnyvale, CA, USA).

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