Chemical constituents from *Euphorbia stracheyi* and their biological activities

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**Abstract**

Three new diterpenoids, stracheyioids A–C (1–3), as well as 36 known compounds (4–39) were isolated from the whole plants of *Euphorbia stracheyi*. Compound 1 was a rare 13-deoxy tigliane diterpenoid and compound 2 was an ingenol diterpenoid characterized by an unique 2Z,4Z-decadienoyl acidic moieties. All the known compounds were isolated from *E. stracheyi* for the first time. Their structures were elucidated on the basis of extensive spectroscopic interpretation. Compounds 1–39 were tested for their cytotoxicity against five cancer cell lines (A-549, MCF-7, Hep G2, Hela and P388) and showed IC\(_{50}\) values in the range 6.64–42.86 \(\mu\)M. The antiangiogenic activities of the isolated compounds were also evaluated using a zebrafish model.

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1. Introduction

*Euphorbia* species are widely distributed plants and were famous for the chemical diversity of their isoprenoid constituents, especially diterpenoids with different core frameworks and variety activities [1]. Many of the plants in genus *Euphorbia* are used in folk medicine but they have not only therapeutic relevance but that they also display toxicity. Studies have suggested that diterpenoids especially those with tigliane, ingenane and abietane skeletons most relevant to the toxicity and considerable biological activities [2].

*Euphorbia stracheyi* Boiss (Euphorbiaceae) was noxious weed distributed in alpine meadow, and in Chinese traditional medicine, its roots were used for hemostasis, analgesia and muscular regeneration [3]. Previous phytochemical investigations on *E. stracheyi* showed that it contained flavonoids, phenolic compounds, ionones, steroids and aliphatic alcohol [3,4]. As part of our program to discover anticancer agents from the genus *Euphorbia* [5,6], a phytochemical investigation on this plant led to the isolation and characterization of three new diterpenoids (1–3) and 36 known compounds including 4 ionones (4–7), 2 sesquiterpenes (8 and 9), 15 diterpenoids (10–24), 1 triterpenoid (25), 1 steroid (26), 1 monosaccharide (27), 9 phenolic compounds (28–36), 1 coumarin (37) and 2 flavonoids (38 and 39) (Fig. 1). Compound 1 was the first natural 12,13-dideoxyphorbol and compound 2 was an ingenol diterpenoid characterized by an unique 2Z,4Z-decadienoyl acidic moieties. Herein, we report the isolation and structure elucidation of those compounds, as well as their cytotoxicity against a small panel of cancer cell lines and antiangiogenic activities obtained using a zebrafish model. The diterpenoids were reported in *E. stracheyi* for the first time and the prominent toxicity of them may one of reasons why *E...
stracheyi is a noxious weed. The phenomena of the biological tests in this study also hint that this diterpenoids maybe play an important role in the chemical defense of the plant E. stracheyi.

The phorbol esters such as TPA (12-O-tetradecanoylphorbol-13-acetate) are known to be powerful tumor promoters and activators of protein kinase C (PKC). Numerous structure-activity analyses of the activators have been carried out and many pharmacophore models have been proposed; however, it has been shown that the biological activity of 13-deoxyphorbol derivatives were not fully consistent with the pharmacophore

Fig. 1. Structures of compounds 1–39.
models so far [7]. Thus, the discovery of first natural 12,13-dideoxyphorbol (1) are interesting and might be helpful to study their structure and unusual biology activity.

2. Experimental

2.1. General

ORD spectra were recorded on a Horiba SEPA-300 polarimeter. UV data were obtained on a Shimadzu UV-2401PC spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. The 1D and 2D NMR experiments were recorded on Bruker AM-400, DRX-500 or Avance III 600 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. ESIMS were recorded using a Finnigan MAT 90 instrument. EI and HREIMS were performed on a Waters AutoSpec Premier P776. Column chromatography (CC) was performed on Sephadex LH-20, silica gel (200–300 mesh, Qingdao Marine Chemical inc., Qingdao, PR China), RP-18 gel (LChroprep, 40–63 μm; Merck, Darmstadt, Germany) and MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semipreparative HPLC was performed on a Hewlett-Packard instrument (column: Zorbax SB–C18, 250 × 9.4 mm; DAD detector). Fractions were monitored by TLC and visualized by heating plates sprayed with 15% H2SO4 in EtOH.

2.2. Plant material

The whole plants of *E. stracheyi* were collected from Segrila Mountain in Nyingchi of the Tibetan Autonomous Region of China in September 2011. A voucher specimen (Yangyp-20110918) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, which was identified by one of the authors (Prof. Yong-Ping Yang).

2.3. Extraction and isolation

The air-dried and powdered whole plants of *E. stracheyi* (1.5 kg) were extracted with 90% EtOH (4 × 6 L) for 24 h at room temperature and concentrated in vacuo to give a crude extract. The extract was suspended in H2O and then extracted with EtOAc. The EtOAc fraction (103 g) was subjected to MCI gel CC (MeOH–H2O, 20:80 to 100:0) in a gradient to afford nine fractions (Fr.1–9). Fr.1 was subjected to Sephadex LH-20 CC (CHCl3–MeOH, 1:1) to obtain 39 (26.9 mg). Fr.2 was repeatedly subjected to silica gel CC eluting with CHCl3–MeOH (10:1 to 4:1) and then purified by Sephadex LH–20 CC (CHCl3–MeOH, 1:1) to afford 27 (15.4 mg), 30 (10.5 mg), 31 (4.0 mg), 33 (6.4 mg), 34 (13.7 mg), 35 (3.2 mg), 36 (12.4 mg), 37 (2.6 mg) and 38 (9.2 mg). Fr.3 was repeatedly subjected to silica gel CC eluting with CHCl3–MeOH (5:1) and then purified by Sephadex LH–20 CC (CHCl3–MeOH, 1:1) to yield 4 (10.3 mg), 5 (2.6 mg), 6 (4.1 mg), 7 (2.5 mg), 18 (43.3 mg) and 24 (4.5 mg). Fr.5 was subjected to silica gel CC eluting with CHCl3–acetone (4:1) to afford 28 (2.6 mg), 29 (19.7 mg) and 32 (1.7 mg). Fr.6 was purified by Sephadex LH–20 CC (CHCl3–MeOH, 1:1) followed by preparative TLC with CHCl3–EtOAc (4:1) to give 8 (4.7 mg) and 9 (1.9 mg). Fr.7 was subjected to silica gel CC eluting with CHCl3–EtOAc (9:1 to 4:1) to yield three major subfractions (Fr.7a–7c). Fr.7a was purified by semipreparative HPLC (MeOH–H2O, 88:12) to afford 3 (2.1 mg) and 10 (2.8 mg). Fr.7b was subjected to Sephadex LH–20 CC (CHCl3–MeOH, 1:1) to obtain 15 (35.7 mg) and 19 (180.3 mg). Fr.7c was subjected to Sephadex LH–20 CC (CHCl3–MeOH, 1:1), followed by
preparative TLC with CHCl₃–EtOAc (4:1), to give 1 (2.2 mg), 11 (4.9 mg) and 12 (3.4 mg). Fr.8 was subjected to silica gel CC eluting with CHCl₃–EtOAc (10:1 to 4:1), to afford four major subfractions (Fr.8a–8d). Fr.8a was subjected to a Sephadex LH-20 CC (CHCl₃–MeOH, 1:1) to afford 25 (40.2 mg) and 26 (2.8 mg). Fr.9 was subjected to silica gel CC eluting with petroleum ether–EtOAc (10:1 to 3:2) to afford three major subfractions (Fr.9a–9c). Fr.9a was subjected to Sephadex LH-20 CC (CHCl₃–MeOH, 1:1) to afford 25 (40.2 mg) and 26 (2.8 mg). Fr.9b was purified by RP-18 CC (MeOH–H₂O, 70:30 to 95:5) in a gradient and then subjected to silica gel CC eluting with petroleum ether–EtOAc (10:1 to 3:1), to afford 16 (125.6 mg) and 22 (32.7 mg).

2.4. Spectroscopic data

2.4.1. Stracheyioid A (1)

White amorphous powder; [α]D −220.6 (c 0.14, MeOH); UV (MeOH) λmax (log ε) 201 (3.98), 240 (3.91) nm; IR (KBr) νmax 3418, 2922, 2858, 1706, 1634, 1616, 1455, 1436, 1382, 1346, 1125, 1056, 1026, 966, 944 cm⁻¹; 1H NMR (DMSO-d₆, 500 MHz) and 13C NMR (DMSO-d₆, 125 MHz)

Table 1

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OH-4 5.17 s
OH-9 4.07 s
OH-16 4.44 s
OH-20 4.66 s

a 1H and 13C NMR data measured at 500 and 125 MHz.
b 1H and 13C NMR data measured at 600 and 150 MHz.
data, see Table 1; positive ESIMS m/z 371 [M + Na]⁺; HREIMS m/z 348.1922 [M]⁺ (calcd for C20H26O5, 348.1937).

2.4.2. Stracheyiod B (2)
Colorless oil; [α]D 15 + 48.0 (c 0.12, MeOH); UV (MeOH) λmax (log ε) 203 (4.46) nm; IR (KBr) νmax 3440, 2956, 2927, 2871, 1729, 1642, 1461, 1380, 1156, 1034, 987 cm⁻¹; 1H NMR (CDCl₃, 600 MHz) and 13C NMR (CDCl₃, 150 MHz) data, see Table 1; positive ESIMS m/z 605 [M + Na]⁺; HREIMS m/z 582.3182 [M]⁺ (calcd for C34H46O8, 582.3193).

2.4.3. Stracheyiod C (3)
White amorphous powder; [α]D 19 + 629.0 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 279 (4.57) nm; IR (KBr) νmax 3482, 2925, 2850, 1747, 1661, 1385, 1366, 1036, 1016 cm⁻¹; 1H NMR (CDCl₃, 600 MHz) and 13C NMR (CDCl₃, 150 MHz) data, see Table 1; positive ESIMS m/z 339 [M + Na]⁺; HREIMS m/z 316.2030 [M]⁺ (calcd for C20H28O5, 316.2038).

2.5. Cytotoxicity assay
Compounds 1–39 were tested for their cytotoxicity against human lung carcinoma (A-549), human breast adenocarcinoma (MCF-7), human hepatocellular carcinoma (Hep G2), human cervical carcinoma (Hela), mouse leukemia (P388) by the MTT method, 5-FU was used as a positive control. Briefly, 100 μL of cell suspension (1 × 10⁵ cells/mL) was seeded into 96-well microtiter plates and cultured for 24 h before the compound was added. Then different concentrations of the compounds were added to the plates, the cells were cultivated for 48 h, and 10 μL of MTT (5 mg/mL) was added to each well. After 4 h, the culture medium was removed and the formazan crystals were completely dissolved with 150 μL DMSO in each well by vigorously shaking the plate. Finally, formazan absorbance was assessed by a BioRad microplate reader at 570 nm.

2.6. Antiangiogenesis assay
Stock solutions (20 mg/mL) of all samples were prepared by dissolving the test compounds in 100% DMSO. These solutions were diluted in sterile salt water (5 mM NaCl, 0.17 mM KCl, 0.4 mM CaCl₂, 0.16 mM MgSO₄) to obtain final solutions of various concentrations in 0.2% DMSO. Aliquots were placed into 24-well plates, and the embryos (TG[VEGFR2: GrCFP]) at 24 hpf (hours post-fertilization) were also transferred randomly into the above wells. Control embryos were treated with the equivalent amount of DMSO solutions. All embryos were incubated at 28.5 °C. After 48 h treatment, the intersegmental vessels of embryos were visualized with green fluorescent protein labeling and endogenous alkaline phosphatase staining. The antiangiogenic activities of compounds were calculated from the inhibition ratio of antiangiogenesis.

3. Results and discussion
Stracheyiod A (1) was obtained as a white amorphous powder. The molecular formula of 1 was determined as C20H26O5 by HREIMS, requiring 7 degrees of unsaturation. The IR spectrum showed absorption bands for OH (3418 cm⁻¹) ketone (1706 cm⁻¹) and double-bond (1634 cm⁻¹) moieties. Analysis of the 1H and 13C NMR (Table 1) data of 1 aided by HSQC revealed resonances for one ketone (δc 209.7), two pair of trisubstituted double bonds (δh 5.11 (1H, s), δc 138.2 (d), δh 130.2 (s); δh 7.31 (1H, s), δc 182 (s), δh 157.2 (d)), four methylenes including two hydroxylated ones (δh 3.01, 3.05 (2H, AB q, J = 10.5 Hz), δc 71.0 (t); δh 3.54, 3.60 (2H, AB q, J = 13.4 Hz), δc 66.4 (t)), two oxygenated quaternary carbons (δc 73.8, 77.5), three methyls [one of which connected to a methine, δh 0.86 (3H, d, J = 6.3 Hz)], five methines, and six quaternary carbon. These signals required 1 to be tetracyclic backbone. Its 1H and 13C NMR spectra showed similarities to those of 12-deoxy-16-hydroxyphorbol [8] revealed that 1 may be a tigiane diterpenoid. The differences were that the carbon signals corresponding to a quaternary carbon (C-13) in 12-deoxy-16-hydroxyphorbol were replaced by a non-oxygenated methine (δc 16.2, C-13) in 1. This deduction was confirmed by the COSY correlation of H-12/H-13/H-14 and the HMBC correlation of H-13/C-11, C-15, C-16; Me-17/C-13 (Fig. 2). In the ROESY spectrum of 1 (Fig. 3), key correlations were observed between H-10/Me-18, OH-9; H-13/H-14; H-16/H-13, H-14 and H-8/Me-17, indicating the α-orientation of OH-9, H-10, Me-18, H-13, H-14 and H-16 and the β-orientation of H-8 and Me-17, the same relative configuration with those of reported [8]. Therefore, the structure of 1 was determined as 12,13-dideoxy-16-hydroxyphorbol, and it is the first example of natural 12,13-dideoxyphorbol despite some 13-deoxyphorbols have been synthesized [7].

Fig. 2. Selected HMBC (--) and 1H-1H COSY ( ) correlations of compounds 1–3.
Stracheyioid B (2) was obtained as optically active colorless oil ([α]D 23 150.0, 0.12), and the molecular formula was deduced to be C34H46O8 based on its HREIMS (m/z 582.3182), suggesting 12 degrees of unsaturation. The IR spectrum of 2 suggested characteristic bands of hydroxyl (3440 cm⁻¹), carboxyl (1729 cm⁻¹) and olefinic (1642 cm⁻¹) groups. Analysis of the NMR data of 2 (Table 1) suggested the presence of one ketone (δC 205.7), four oxygen-bearing carbons [δC 66.3 (t), 77.0 (d), 82.4 (d), 85.9 (s)], three ester carbonyls (δC 165.8, 170.7, and 172.6), four pair of double bonds, six methylenes and seven methyls. Comparison of the 1D NMR spectra (Table 1) of 2 with those of 3,20-O-diacetyligenol-5-O-(2′E,4′Z)-tetradecadienoate [9] revealed that 2 may be an ingenol triester. The differences between them could be rationalized to the carbon signals corresponding to acid moieties. The significant EIMS fragment peaks at m/z 151 [C6H15COOH]+, 414 [M–C6H15COOH]+, 354 [M–C6H15COOH–CH3COOH]+, 294 [M–C8H15COOH–2×CH3COOH]+ suggested the acidic moieties of 2 were 2,4-decadienoyl and two acetyls. The HMBC (Fig. 2) correlations of H-3/C-1′, H-20/C-1″ demonstrated that two acetyls were located at C-3 and C-20, respectively. The proton signal of H-5 in 2 resonated at δ 5.43 (s), shifting downfield by 1.74 ppm compared to those ingenols in which 5-OH was non-acylation (δH 4.40, 2H, s), one oxymethine (δH 4.94, 1H, dd, J = 13.5, 6.3 Hz) and one olefinic methine (δH 6.38, 1H, s). Twenty carbon resonances (6 × C, 4 × CH, 7 × CH2, 3 × CH3) appeared in the 13C NMR spectrum, and two oxygenated carbons (δC 55.2 (t), 76.5 (d)). A comparison of the NMR data of 3 with those of jolkinolide E [13] indicated that they were very similar except that a hydroxyl group was substituted at C-17, which was confirmed by HMBC correlations of H-17 with C-13, C-15 and C-16 (Fig. 2). The ROESY correlations (Fig. 3) of H-5/H-9, Me-18; Me-20/H-12, Me-19 suggested that compound 3 sharing the relative configuration with the reported. As a result, the structure of stracheyioid C (3) was determined as 17-hydroxy-ent-abieta-8(14),13(15)-dien-16,12-olide.

The known compounds were identified as byzantionioside B [4] [14], (6R,9S)-3-oxo-α-ionol-β-d-glucopyranoside [5] [15], matenosides B [6] [16], icariside B2 [7] [17], 9β-hydroxycostus acid [8] [18], isolantolactone [9] [19], ent-11β-hydroxyabieta-8(14),13(15)-dien-16,12-olide [10] [20], (2R,3S,4R,5R,9S,11S,15R)-3,5,15-O-triacyl-14-oxalolyl-6(17),12-diene [11] [21], langduin A [12] [22], 3-O-(2′E,4′Z-decadienoyl)-20-O-tetradecanoyl-ingenol [13] [23], sikkimenoe E [14] [24], prostratin [15] [25], 13-O-tetradecanoyl-12-deoxyphorbol [16] [26], 13-O-tetradecanoyl-20-O-acetyl-12-deoxyphorbol [17] [27], fischeroside A [18] [28], 13-O-acetylfphotosphorbol [19] [29], 12-O-tetradecanoylphorbol [20] [30], 12-O-tetradecanoyl-13-O-acetylfphotosphorbol [21] [31], 12-O-tetradecanoyl-13,20-O-diacetylfphotosphorbol [22] [32], 12-O-(2′E,4′E-decadienoyl)-13-O-acetylfphotosphorbol [23] [33], fischeroside C [24] [28], 3β-hydroxy-cycloart-23-ene-25-methyl ether [25] [34], 3β-hydroxy-4α, 14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one [26] [35], ethyl glucopyranoside [27] [36], methyl gallate [28] [37], ethyl gallate [29] [37], 4-methoxybenzyl-3β-hydroxyd-glucopyranoside [30]
[38], salidroside (31) [39], 3-hydroxybenzeneethanol (32) [40], 1-O-β-D-glucopyranosyl-2-(3-hydroxyphenyl) ethanol (33) [41], 4-O-glycosylxy-2-hydroxy-6-methoxyacetophenone (34) [42], coniferin (35) [43], neochlorogenic acid methyl ester (36) [44], eleutheroside B1 (37) [45], quercetin-3-O-β-D-galactoside (38) [46] and quercetin-3-O-(3′′-O-galloyl)-β-D-galactopyranoside (39) [47] by comparison of their experimental and reported spectroscopic data. All the compounds were isolated from E. stracheyi for the first time.

Since ligulane, ingenane and abietane diterpenoids were the main anticancer substances from genus Euphorbia [2], the cytotoxicity of compounds 1–39 were evaluated against human lung carcinoma (A-549), human breast adenocarcinoma (Hela) and mouse leukemia (P388) cell lines by MTT cytotoxicity of compounds and [41],4 -

Fig. 4. The impacts of compound 11 on zebrafish embryos. (A) Control embryos (48 hpf); (B) embryonic pericardial edema appeared after treated with compound 11 (1 μg/mL) for 24. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

References