A new tigliane-type diterpene from *Euphorbia dracunculoides* Lam

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ABSTRACT
One new tigliane-type diterpene, 4-deoxy-4(β)H-8-hydroperoxyphorbol-12-benzoate-13-isobutyrate (1), together with two known diterpenoids, 3-acetyl-5,8-dibenzoyl-14\textalpha-propanoyl-13,17-epoxy-7-myrismaone diterpene with C9–C10 cyclised to form an additional lactone ring (2), Euphodendriane A (3) have been isolated from the whole plants of *Euphorbia dracunculoides* Lam. Their structures were elucidated by means of extensive spectroscopic analysis (NMR and HR-ESI-MS) and comparison with data reported in the literature. This is the first isolation of 8-hydroperoxy tigliane diterpene (1) from the genus of *Euphorbia*. All compounds were evaluated for their antifungal activities.

1. Introduction

*Euphorbia* is the largest genus of the plant family Euphorbiaceae, with approximately 2160 species. Many plants from the genus *Euphorbia* are used as a folk medicine for the treatment of skin diseases, diarrhoea, oedemas, etc. The major characteristic constituents of the genus are diterpenoids with many different core skeletons, including jatrophanes, lathyranes, tiglianes, daphnane, ingenanes, myrsinols, etc. (Shi et al. 2008; Ferreira et al. 2013; Ghanadian et al. 2013; Rehman et al. 2014; Vasas & Hohmann 2014). *Euphorbia dracunculoides* Lam is a perennial herb mainly distributed in Tropical African, South Europe and South-west Asia. The plant has been used ethnobotanically for its purgative and diuretic purposes in India.
Previous phytochemical studies on *E. dracunculoides* Lam led to the isolation of a number of structurally diverse diterpenoids, triterpenoids, etc. (Wang, Cao, et al. 2015; Wang, Ma, et al. 2015; Wang, Yang, et al. 2015; Wang, Zang, et al. 2015). In this study, one new tigliane-type diterpene (1), together with two known diterpenoids (2 and 3) (Figure 1) has been isolated from *E. dracunculoides* Lam. All isolated metabolites (1–3) were evaluated for their antifungal activities against *Candida albicans*. Herein, we report the isolation, structure elucidation and antifungal activities of isolated compounds.

2. Results and discussion

Compound 1 was obtained as an amorphous white solid. Its molecular formula was determined to be \( \text{C}_{31}\text{H}_{38}\text{O}_9 \) by high resolution ESI-MS (m/z 577.2407 Calcd for [M + Na]^+, 577.2408) and 31 carbon signals were observed in the \(^{13}\text{C}\) NMR spectrum, indicating 13 degrees of unsaturation. A DEPT NMR experiment permitted differentiation of the 31 resonances into 7 methyls, 14 methines and 10 quaternary carbons. The \(^1\text{H}\) NMR spectrum of 1 in methanol-d\(_4\) displayed that the 7 methyl group resonating at \( \delta^\text{H} \) 1.18, 1.29, 0.72, 1.72, 1.43, 1.15, and 1.14 (3H each), which are correlated with the carbons at \( \delta^\text{C} \) 24.3 (C-16), 19.2 (C-17), 15.2 (C-18), 10.3 (C-19), 23.9 (C-20), 19.2 (C-3") and 19.1 (C-4") respectively, in the HMQC spectrum. Analysis of its \(^{13}\text{C}\) NMR and DEPT spectrum of 1 showed that the three carbonyl carbons at \( \delta^\text{C} \) 211.8 (C-3), 168.1 (C-1") and 180.7 (C-1”). The \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectrum contains aromatic signals at \( \delta^\text{H} \) 7.96 (H-3’, 7’), 7.47 (H-4’, 6’), 7.59 (H-5’) and \( \delta^\text{C} \) 131.4 (qC, C-2”), 130.8 (CH, C-3’, 7’), 129.8 (CH, C-4’, 6’), 134.6 (CH, C-5”), which indicates the presence of a benzoate moiety. This was further supported by HMBC correlations between aromatic proton at \( \delta^\text{H} \) 7.96 (H-3’, 7’), 7.47 (H-4’, 6’) with carbonyl carbon at \( \delta^\text{C} \) 168.1 (C-1”). The presence of isobutyrate moiety in 1 was evident from the \(^1\text{H}–^1\text{H}\) correlations of 1.15 (3H, Me-3")/2.56 (2H, H-2") and 1.14 (3H, Me-4")/2.56 (2H, H-2") in the COSY spectrum. This also was further supported by the long range H–C correlations of 1.15 (3H, Me-3”), 1.14 (3H, Me-4”) with carbonyl carbon at \( \delta^\text{C} \) 180.7 (C-1”), respectively, in the HMBC spectrum. There are five methyls (\( \delta^\text{C} \) at 24.3, 19.2, 15.2, 10.3, 23.9), eight methines (two oxygenated carbons \( \delta^\text{C} \) at 71.2, 79.8 and two olefinic carbons \( \delta^\text{C} \) at 164.2, 133.5) and seven quaternary carbons (three oxygenated carbons \( \delta^\text{C} \) at 87.7, 78.7, 79.8, two olefinic carbons \( \delta^\text{C} \) at 140.5, 141.7 and one ketone carbonyl carbon \( \delta^\text{C} \) at...
211.8) in the remaining 20 carbons signals of 1. The above characteristic data suggested that compound 1 possessed a phorbol-type skeleton of the tigliane-type diterpenoids, which could be supported by its DEPT, $^1$H–$^1$H COSY, HMQC and HMBC experiments (Wang, Wang, et al. 2015). The NMR data of 1 were closely similar to those of another known tigliane diterpene, Euphodendriane A (Aljancic et al. 2011), except for C-8 which was shifted downfield at $\delta_C$ 87.7 ppm. On the base of its molecular formula, one oxygen atom still remains in the molecule, and the distinct downfield chemical shift of the oxygenated carbon C-8 resonance at $\delta_C$ 87.7, the presence of a hydroperoxy moiety at C-8 was determined (Asada et al. 2013; Corlay et al. 2014). The 2D NMR spectra were in turn used to determine the linkage of the benzoate moiety to tigliane-type diterpenoid skeleton. The critical long-range correlation between the methine proton at 5.69 (H-12) and carbonyl carbon at $\delta_C$ 168.1 (C-1’) indicated that benzoate unit was linked to carbon at 79.8 (C-12). The isobutyrate moiety was located at 68.0 (C-13) by comparing to characteristic chemical shift of C-13 ($\delta_C$ 68.0) at and carbonyl carbon ($\delta_C$ 180.7, C-1”) with similar tigliane derivatives (Marco et al. 1999; Aljancic et al. 2011; Wang, Wang, et al. 2015). The ROESY spectrum was employed to determine the relative conformation of 1. All tigliane diterpenoids possess H-8$\beta$, OH-9$\alpha$ and H-10$\alpha$ configurations (Aljancic et al. 2011). The $\alpha$-orientation of Me-16 ($\delta_C$ at 24.3) was elucidated by comparison of its NMR spectroscopic data with related tigliane compounds (Aljancic et al. 2011; Asada et al. 2013; Wang, Wang, et al. 2015). The NOE correlation of 1.93 (H-11)/1.29 (Me-17) confirmed $\beta$-configuration of H-11. The NOE correlation of 2.67 (H-4)/1.93 (H-11$\beta$) revealed that H-4 was $\beta$-oriented. The NOE correlation was observed from 4.65 (H-5) to 0.72 (Me-18), indicating that H-5 was $\alpha$-orientation. The strong NOE correlation between 0.72 (Me-18)/5.69 (H-12) and a relatively large coupling constant ($J = 10.0$ Hz) between H-11$\beta$ and H-12 indicate $\alpha$-configuration of H-12. The NOE correlations of 1.18 (Me-16)/2.06 (H-12), and 1.18 (Me-16)/2.56 (H-2”) indicated $\alpha$-orientation of both H-12 and isobutyrate moiety. The full $^1$H and $^{13}$C NMR assignments were established by means of a combination of COSY, HMQC, HMBC and ROESY spectral measurements (Supporting material Table S1 and Figure S15). Thus, the structure of 1 was established as 4-deoxy-4($\beta$)H-8-hydroperoxyphorbol-12-benzoate-13-isobutyrate. To the best of our knowledge, compound 1 is first 8-hydroperoxy tigliane diterpene example. It is notable that hydroperoxy-phorbol compounds are rare and only seven analogues are isolated from nature resource (Asada et al. 2013; Corlay et al. 2014; Wang, Wang, et al. 2015). This is also first isolation of hydroperoxy-phorbol derivative from Euphorbia genus.

The known compounds were identified by comparison of their NMR and HR-ESI-MS spectroscopic data with those reported in the literature as 3-acetyl-5,8-dibenzoyl-14$\alpha$-propanoyl-13,17-epoxy-7-myrsinaone diterpene with C9–C10 cyclised to form an additional lactone ring (2) (Zhang et al. 1998), Euphodendriane A (3) (Aljancic et al. 2011). It should be mentioned that compound 2 is isolated from E. dracunculoides Lam for the first time.

All isolated metabolites (1–3) were found to be inactive at the highest test concentration of 128 $\mu$g/mL used against the pathogenic fungi C. albicans.

3. Experimental

3.1. General

The 1D and 2D NMR (COSY, HMQC, HMBC, ROESY) spectra using standard pulse programs were recorded at room temperature on a Bruker Avance DRX 500 FT spectrometer (Bruker,
Karlsruhe, Germany) operating at 500 (1H) or 125 (13C) MHz. The chemical shift values are relative to the internal standard TMS. HR-ESI-MS data were obtained on an Agilent Series 1100 SL mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Preparative HPLC was performed on an Agilent 1260 Chromatograph equipped with a G1311C pump, G1315D photodiode array detector at 254 nm and Agilent-Pack ODS column (250 × 9.4 mm, 5 μm, Agilent Technologies, Santa Clara, CA, USA). Column chromatography was performed using normal phase silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China) and reversed phase silica gel (RP-18, J.T. Baker, 40 μm, Merck, Darmstadt, Germany), TLC was carried out on silica gel sheets (Qingdao Marine Chemical, Inc., Qingdao, China) and reversed phase plates (RP-18 F254S, Merck, Darmstadt, Germany). Visualisation: UV at 254 nm or 10% H2SO4 followed by heating.

3.2. Plant material

The whole plants of *E. dracunculoides* Lam were collected in Lijiang, Yunnan Province, China, in July, 2012, and identified by Prof Fan Du. A voucher specimen (No. 120701) is deposited in the Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Southwest Forestry University, Kunming City, Yunnan Province, China.

3.3. Extraction and isolation

Powdered, air-dried whole plants of *E. dracunculoides* Lam (8.2 kg) were exhaustively extracted with 95% EtOH (45 L × 3) at room temperature for 72 h. The extraction was evaporated to dryness in vacuo. The residue (1300 g) was suspended in H2O and then partitioned with petroleum ether and EtOAc, respectively. The EtOAc layer was evaporated to dryness and the resultant residue (130.0 g) was subjected to silica gel liquid chromatography (80 × 810 mm) using stepwise gradient elution CHCl3/MeOH at 100:0 (3000 ml), 50:1 (3500 ml), 20:1 (3750 ml), 10:1 (5200 ml), 7:1 (5800 ml), 5:1 (9000 ml), 3:1 (2300 ml), 2:1 (1500 ml) and finally with MeOH (2900 ml) to afford 14 pooled fractions A-N according to TLC. Fraction D (11.3 g) was purified on silica gel column chromatography (40 × 770 mm) eluting with petroleum ether/acetone at 60:1 (2000 ml), 40:1 (2000 ml), 20:1 (2500 ml), 10:1 (3800 ml), 5:1 (2600 ml), 3:1 (2300 ml), 2:1 (1500 ml) to give 15 pooled fractions (1–15). Fraction 10 (1.1 g) further purified on a C18 reversed phase column (40 × 570 mm) eluting with MeOH/H2O at 50:50 (1200 ml), 60:40 (960 ml), 70:30 (960 ml), 80:20 (2045 ml), 90:10 (1940 ml), 100:0 (1800 ml) to give 10 pooled fractions (J1–J10). Fraction J6 (110.0 mg) was chromatographed on a semipreparative C18 reversed phase HPLC column, using 74% MeOH/H2O, at a flow rate of 4 mL/min, to give compounds 1 (13.0 mg, tR 14.8 min), 2 (12.0 mg, tR 18.6 min) and 3 (4.0 mg, tR 25.8 min).

3.4. Spectroscopic data

4-deoxy-4(β)H-8-hydroperoxyphorbol-12-benzoate-13-isobutyrate (1): colourless amorphous powder, ESI-MS at 555 for [M + H]+, 577 for [M + Na]+, HR-ESI-MS found at m/z 577.2407 Calcd for [M + Na]+, 577.2408; 1H (500 MHz) and 13C (125 MHz) NMR data for compound 1 in Methanol-d4 (δ ppm, J in Hz). 1H-NMR: 7.79 (1H, s, H-1), 2.67 (1H, d, J = 4.0, 2.0 Hz, H-4), 4.65 (1H, s, H-5), 5.26 (1H, t, J = 1.5 Hz, H-7), 3.65 (1H, m, H-10), 1.93 (1H, m, H-11), 5.69 (1H, d,
In this study, a new tigliane-type diterpene, 4-deoxy-4(β)-H-8-hydroperoxyhorbol-12-benzoate-13-isobutyrate (1), together with two known diterpenoids (2–3), has been isolated.
from the whole plants of *E. dracunculoides* Lam. The compound, 8-hydroperoxy tigliane diterpene (1), was isolated from the genus of *Euphorbia* by the first time. All isolated metabolites (1–3) were found to be inactive at the highest test concentration of 128 μg/mL used against the pathogenic fungi *C. albicans*.

**Supplementary material**

Supplementary material relating to this article are available online, alongside Figures S1–S15 and Table S1.

**Acknowledgements**

The authors thank Mr Run-Xi He for providing plant material.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by the Natural Science Foundation of China (NSFC) [grant number 31160075, [grant number 21362035]; The Initial Foundation of Scientific Research for the introduction of talents from Southwest Forestry University for Wen-Hui Xu [grant number 20130916]; Sci-tech Innovation Foundation of Southwest Forestry University [grant number C1478].

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