A new cytotoxic casbane diterpene from *Euphorbia pekinensis*

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**ABSTRACT**

A new cytotoxic casbane diterpene, named pekinenal, was isolated from the roots of *Euphorbia pekinensis*. Its structure was elucidated as 5α-hydroxy-1βH,2αH-casba-3Z,7E,11E-triene-18-al by a combination of 1D- and 2D-NMR techniques and confirmed by X-ray crystallography. Pekinenal showed cytotoxic activity against all four human cancer cell lines tested.

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**Keywords:**

Euphorbia pekinensis
Casbane diterpene
Pekinenal
5α-hydroxy-1βH,2αH-casba-3Z,7E,11E-triene-18-al
Cytotoxic
X-ray

1. Introduction

Euphorbia, the largest genus of Euphorbiaceae, with more than 2000 species is characterized by the presence of milky latex [1]. There are five species, *E. pekinensis*, *E. kansui*, *E. lathyris*, *E. humifusa*, and *E. maculata* that have been recorded in Chinese pharmacopoeias for the treatment of oedema, gonorrhea, migraine and wart cures, whereas they are well-known poisonous plants. Modern phytochemical and pharmacological studies have shown that this genus produces various bioactive, structurally unique macro- and polycyclic diterpenes. Some of them possess cytotoxic, modulability of multidrug resistance, antiviral and anti-inflammatory activity as well as skin-irritant, tumor promoting, and proinflammatory properties [2–8]. To our best knowledge, only a few phytochemical studies have been published on the roots of *E. pekinensis* to date [9]. In this paper, we describe the isolation and structural elucidation of a new casbane diterpene, pekinenal (1) (Fig. 1) from the title plant based on spectral data and single-crystal X-ray analysis and its cytotoxic activities against human cancer cell lines.

2. Experimental

2.1. General

Melting point was determined on an X4 micro-melting point apparatus and was uncorrected. Optical rotation was measured on a Perkin-Elmer model 241 polarimeter. IR spectrum was obtained on a Nicolet Impact infrared spectrophotometer with KBr pellets. NMR spectra were recorded on a Bruker DRX-400 spectrometer. HR-ESIMS was obtained on an Agilent 110 MSD mass spectrometer. Column chromatography (CC) was made on silica gel (100–200 mesh and 200–300 mesh, Marine Chemical Factory in Qingdao); TLC was performed on precoated silica gel plates (HSG and HSF254, Yantai Chemical Factory), and spots were detected by spraying with vanillin–sulphuric acid reagent, followed by heating.

2.2. Plant

The roots of *Euphorbia pekinensis* were collected from the suburbs of Nanjing (Jiangsu, China) in September 2006 and authenticated by Prof. Cao Jianguo, Nanjing University of Chinese Medicine. A voucher specimen (No. 20060923) is

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deposited at the Herbarium of Nanjing University of Chinese Medicine in China.

2.3. Extraction and isolation

Dried powdered roots (1 kg) of *E. pekinensis* were extracted (× 3) with 95% EtOH. After filtration and removal of the solvent under reduced pressure, the residue was partitioned between H2O and CHCl3. The CHCl3 extract (57 g) was subjected to a Si-gel CC eluted with petroleum ether and petroleum ether–EtOAc mixtures of increasing polarity. The eluates were monitored by TLC and grouped into 16 fractions (Fr.E1–Fr.E16). Evaporation of Fr. E6 eluted with petroleum ether and EtOAc (92:8) gives a light yellow powder (0.2 g). The CHCl3 extract (57 g) was subjected to preparative TLC and developed with hexane–EtOAc (98:2) to afford 1 (30 mg).

Euphpekinin (1, Fig. 1), colorless needles; mp.122–123 °C; [α]D24 +6.24° (c 0.05, CHCl3); UV λmax (MeOH): 268 nm; IR νmax (KBr): 3420, 2833, 1710, 1665, 1626, 1459, 1378, 1253, and 1038 cm−1; HR-ESIMS m/z [M + H]+ 303.2324; 1H NMR (400 MHz) and 13C NMR (100 MHz) determined by HR-ESIMS exhibiting an ion peak at 303.2357 ([M+H]+, calcd. 303.2324). Its IR spectrum showed the presence of hydroxyl (3420 cm−1), carbonyl (1710 cm−1), and olefin (1670 and 1626 cm−1) groups. The UV spectrum showed a maximum absorption at 268 nm, indicating the presence of an α, β-unsaturated carboxyl group. The molecular formula of 1 required 6° of unsaturation. HMOC and DEPT spectra (Table 1) of 1 displayed the 20 signals corresponding to four methyl, five methylene (all sp3), seven methine (four sp2 and two sp3), and four quaternary (three sp2 and one sp3) carbon atoms, these last attributable to one carbonyl and three olefinic groups. The two remaining degrees of unsaturation were ascribed to diyclic ring system. The 1H,1H COSY of 1 revealed three spin systems, beginning with the proton (δ 0.79, H-1) in the most upfield region of the spectrum. In 1H,13C COSY spectrum, H-1 was coupled with both H-2 (δ 2.07) and a methylene H-13 (δ 1.96). H-2 was further coupled with an olefinic proton (δ 6.06, H-3) and H-14 coupled with a methylene H-13 (δ 2.30, 2.01). The system of coupled protons in this region terminated with the presence of quaternary centers at C-4 and C-12, respectively. The other two coupled units determined by 1H,13C COSY spectrum from H-5 (δ 5.05, linked to hydroxyl group) to H-7 (δ 5.21) and H-9 (δ 2.04, 2.14) to H-11 (δ 5.08) likewise ended with the presence of quaternary carbon atoms at C-4, C-8 and C-12. All the carbon signals in the three spin coupled units were assigned through the HMOC experiment (Table 1) and these units were connected on the basis of HMBC experiment (Fig. 2). In the HMBC spectrum, correlations were observed between C-4 (δ 142.80) and H-6 (δ 2.50, 2.62), C-18 (δ 193.89) and H-3, as well as between C-8 (δ 138.42) and both H-6 and a methyl H-19 (δ 1.52), and both C-19 (δ 16.75) and C-9 (δ 38.70) and H-7. Furthermore, the HMBC experiment yielded correlations between the quaternary carbon C-12 (δ 134.16) and H-10.

### Table 1

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<td>2.62 ddd (9.6, 9.0, 2.5)</td>
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2.4. Cytotoxicity test (MTT colorimetric assay)

The human cancer cell lines were cultivated in humidified incubators (5% CO2 and 37 °C). The cells were grown in an RPMI 1640 medium containing 10% FBS and antibiotics (100 U/ml of penicillin and 0.1 mg/ml of streptomycin). The cells (1 × 105/well) were seeded in 96-well plates and cultivated for 12 h. Then, the cells were treated with compound 1 of six concentrations (2.5, 5, 10, 20, 40, 60 µg/ml) for 48 h. Cytotoxicity was determined as described previously [10].

3. Results and discussion

Pekinenal (1) possessed a molecular formula C20H30O2 determined by HR-ESIMS exhibiting an ion peak at 303.2357 ([M+H]+, calcd. 303.2324). Its IR spectrum showed the presence of hydroxyl (3420 cm−1), carbonyl (1710 cm−1), and olefin (1670 and 1626 cm−1) groups. The UV spectrum showed a maximum absorption at 268 nm, indicating the presence of an α, β-unsaturated carboxyl group. The molecular formula of 1 required 6° of unsaturation. HMOC and DEPT spectra (Table 1) of 1 displayed the 20 signals corresponding to four methyl, five methylene (all sp3), seven methine (four sp2 and two sp3), and four quaternary (three sp2 and one sp3) carbon atoms, these last attributable to one carbonyl and three olefinic groups. The two remaining degrees of unsaturation were ascribed to diyclic ring system. The 1H,1H COSY of 1 revealed three spin systems, beginning with the proton (δ 0.79, H-1) in the most upfield region of the spectrum. In 1H,13C COSY spectrum, H-1 was coupled with both H-2 (δ 2.07) and a methylene H-13 (δ 1.96). H-2 was further coupled with an olefinic proton (δ 6.06, H-3) and H-14 coupled with a methylene H-13 (δ 2.30, 2.01). The system of coupled protons in this region terminated with the presence of quaternary centers at C-4 and C-12, respectively. The other two coupled units determined by 1H,13C COSY spectrum from H-5 (δ 5.05, linked to hydroxyl group) to H-7 (δ 5.21) and H-9 (δ 2.04, 2.14) to H-11 (δ 5.08) likewise ended with the presence of quaternary carbon atoms at C-4, C-8 and C-12. All the carbon signals in the three spin coupled units were assigned through the HMOC experiment (Table 1) and these units were connected on the basis of HMBC experiment (Fig. 2). In the HMBC spectrum, correlations were observed between C-4 (δ 142.80) and H-6 (δ 2.50, 2.62), C-18 (δ 193.89) and H-3, as well as between C-8 (δ 138.42) and both H-6 and a methyl H-19 (δ 1.52), and both C-19 (δ 16.75) and C-9 (δ 38.70) and H-7. Furthermore, the HMBC experiment yielded correlations between the quaternary carbon C-12 (δ 134.16) and H-10.

![Fig. 1.](Q.L. Liang et al. / Fitoterapia 80 (2009) 514–516)

![Fig. 2.](Q.L. Liang et al. / Fitoterapia 80 (2009) 514–516)
(δ 2.18), C-11 (δ 123.58) and both H-13 and H-20 (δ 1.62). The above long-range correlations suggested the presence of a 14 member macrocyclic ring. In addition, correlations were observed between C-2 (δ 34.07) and both two methyls H-16, -17 (δ 1.14, 1.12), as well as between a quaternary carbon C-15 (δ 27.99) and these methyls in the HMBC spectrum, which indicated the presence of cyclopropyl ring bearing the geminal methyl groups. Thus, 1 was a casbane-type diterpenoid. The relative stereochemistry of 1 was determined by NOESY spectrum except C5-OH. The key NOE cross peaks were observed between H1 – H3, H1 – H17, H2 – H16, H3 – H18, H9a – H19, and H10a – H20, whereas NOEs were not observed between H7 – H19 and H11 – H20 (Fig. 2).

X-ray crystallography analysis of 1 gave C5-OH α-oriented (Fig. 3) and confirmed its structure 5α-hydroxy-1βH,2αH-casba-3Z,7E,11E-triene-18-al. The casbane diterpenes are regarded as the precursor of the polycyclic diterpene derivatives occurring widely in the Euphorbiaceae [11]. But casbane diterpenes are rare in the plant kingdom and have only been found so far in 9 species belonging to 6 genera of the family Euphorbiaceae [12–19].

Some of casbane diterpenes possess antibacterial, antitumor and cytotoxic activity [16–18]. We evaluated the cytotoxic activity of 1 against four human cancer cell lines: NCI-H460 (lung carcinoma), KB (nasopharyngeal carcinoma), SGC7901 (gastric carcinoma), and HO-8910 (ovarian carcinoma) by MTT assay. 1 exhibited cytotoxic activity with IC50 values of 10.05, 8.52, 13.82, and 14.16 µg ml−1, respectively.

### 4. X-ray analysis

Single crystals of compound 1 were prepared from CHCl3–acetone (1:1). They were colorless column crystals, orthorhombic system, space group P212121, with a = 10.316(1) Å, b = 10.703(1) Å, c = 16.813(1) Å, V = 1856.4(6) Å3, and Dcalc = 1.082 g cm−3 for Z = 4 (C20H30O2, mol wt 302.44). Crystal dimensions: 0.15×0.20×0.40 mm. The diffraction maxima were collected on a MAC DIP-2030K image plate detector, using graphite-monochromated MoKα radiation and taking oscillation IP photos: 30 frames in total, φ = 0–180°, ∆φ = 6° and 0.9° min−1 for scan rate. A total of 4141 reflections were measured, of which 2678 with |F|2 ≥ 2σ(|F|2) were used for structure determination and refinement. The crystal structure was solved by a direct-solution procedure (shelxs 97) and was refined by least-square methods. The final credible factors are R1 = 0.0485, wR2 = 0.1192, and goodness-of-fit S = 1.027.

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### References