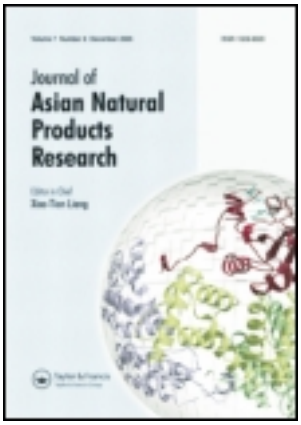

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Two new casbane diterpenoids from the roots of *Euphorbia pekinensis*

Feng-Guang Shao [a b], Ren Bu [b], Cui Zhang [a], Chao-Jun Chen [b], Jian Huang [a c] & Jin-Hui Wang [a b c d]

[a] School of Traditional Chinese Materia Medica 49#, Shenyang Pharmaceutical University, Shenyang, 110016, China

[b] School of Pharmacy, Inner Mongolia Medical College, Hohhot, 010110, China

[c] Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, Shenyang Pharmaceutical University, Shenyang, 110016, China

[d] School of Pharmacy, Shihezi University, Shihezi, 832002, China

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Two new casbane diterpenoids from the roots of *Euphorbia pekinensis*

Feng-Guang Shao[ab], Ren Bu[b], Cui Zhang[a], Chao-Jun Chen[b], Jian Huang[ac] and Jin-Hui Wang[abcd]*

[a]*School of Traditional Chinese Materia Medica 49#, Shenyang Pharmaceutical University, Shenyang 110016, China;* [b]*School of Pharmacy, Inner Mongolia Medical College, Hohhot 010110, China;* [c]*Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China;* [d]*School of Pharmacy, Shihezi University, Shihezi 832002, China*

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From the roots of *Euphorbia pekinensis*, two new casbane diterpenoids, named pekinenins A (**1**) and B (**2**), were isolated. Their structures were elucidated as 18-hydroxy-1β*H*,2α*H*-casba-3*E*,7*E*,11*E*-trien-5-one (**1**), 5α-methoxy-1β*H*,2α*H*-casba-3*Z*,7*E*,11*E*-trien-18-oic acid (**2**) by a combination of 1D and 2D NMR spectroscopy and mass spectrometry. In the cytotoxicity assay of the two new compounds against Hela, MCF-7, and C6 human cancer cell lines, compound **1** showed moderate cytotoxic activity against two human cancer cell lines, Hela and C6, with IC_{50} values of 42.97 and 50.00 μM, respectively.

Keywords: *Euphorbia pekinensis* Rupr; pekinenin A; pekinenin B; casbane diterpenoids

1. Introduction

Diterpenoids are important constituents of the medicinal plant, *Euphorbia pekinensis*, belonging to the family Euphorbiaceae. In traditional Chinese medicine, this plant is used to treat gonorrhea, edema, migraine, and warts [1]. Previous studies on this plant have led to the isolation of diterpenoids, triterpenoids, and flavanoids [2,3]. To discover more diterpenoids used for the study of the structure–activity relationship, we have investigated the title plant. Herein, we report the isolation and structure elucidation of two new casbane diterpenoids, including pekinenins A (**1**) and B (**2**) (Figure 1), found in the petroleum ether (PE) extract of the roots of *E. pekinensis*. Compounds **1** and **2** were evaluated for their cytotoxicities against the Hela, MCF-7, and C6 human cancer cell lines *in vitro.*

2. Results and discussion

Compound **1** was obtained as a yellow oil. It possessed a molecular formula $C_{20}H_{30}O_2$, determined by HR-ESI-MS at *m/z* 627.4387 $[2M + Na]^+$. Its IR spectrum showed strong absorption bands at 3417, 1750, 1670, and 1626 cm^{-1} ascribed to hydroxyl, carbonyl, and olefin groups. The UV spectrum showed absorption maximum at 269 nm, indicating the presence of an α,β-unsaturated carbonyl group. The molecular formula of **1** required six degrees of unsaturation. The ^{13}C NMR spectrum displayed signals for 20 carbons (Table 1). The ^{13}C NMR and DEPT spectra of **1** indicated the presence of one carbonyl

*Corresponding author. Email: wjh.1972@yahoo.com.cn

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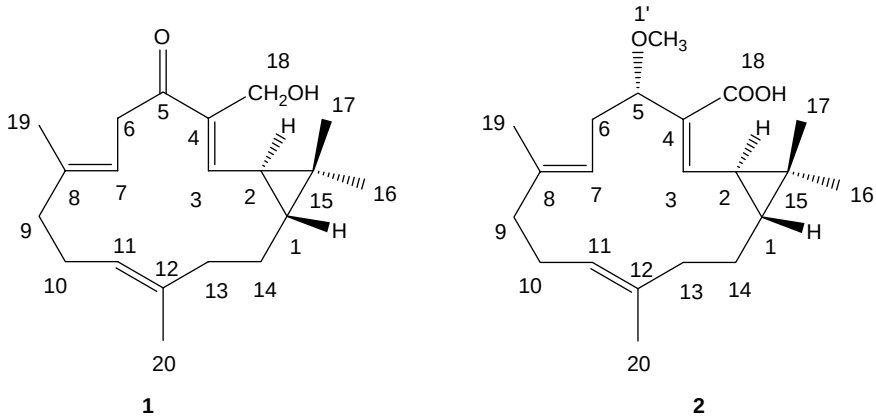

Figure 1. Structures of compounds **1** and **2**.

group, three double bonds, one oxygenated methylene together with 12 aliphatic including four methyl, five methylene, one quaternary, and two methine carbons. The two remaining degrees of unsaturation were ascribed to two carbocyclic systems.

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** in $CDCl_3$ (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR, δ in ppm, J in Hz).

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1	0.79–0.90 (1H, m)	38.3	0.71 (1H, td, $J = 3.9, 11.1$)	36.6
2	1.22–1.36 (1H, m)	31.5	2.00–2.26 (1H, m)	31.0
3	6.22 (1H, d, $J = 10.8$)	152.5	5.58 (1H, d, $J = 10.8$)	152.9
4	–	135.2	–	124.4
5	–	202.5	3.70 (1H, dd, $J = 5.4, 11.1$)	87.1
6	2.84 (1H, d, $J = 13.8$), 3.75 (1H, dd, $J = 11.4, 13.8$)	40.3	2.40–2.55 (1H, m), 2.55–2.70 (1H, m)	31.6
7	5.20 (1H, d, $J = 11.4$)	120.8	4.84 (1H, br s)	119.2
8	–	135.9	–	136.7
9	1.80–2.30 (2H, m)[a]	38.5	1.92–2.10 (2H, m)	38.4
10	1.85–1.95 (2H, m)[a]	24.2	1.80–1.98 (1H, m), 2.00–2.25 (1H, m)[a]	23.9
11	4.95 (1H, d, $J = 6.0$)	125.3	5.02 (1H, br s)	125.2
12	–	133.4	–	132.8
13	1.80–2.30 (2H, m)[a]	38.5	1.90–2.05 (1H, m), 2.20–2.32 (1H, m)	38.9
14	1.98–2.15 (2H, m)[a]	23.9	2.00–2.25 (2H, m)[a]	23.8
15	–	29.2	–	27.0
16	1.15 (3H, s)	23.5	1.11 (3H, s)	21.8
17	1.16 (3H, s)	21.8	1.14 (3H, s)	22.7
18	4.33 (1H, d, $J = 11.4$), 4.40 (1H, d, $J = 11.4$)	57.6	–	167.5
19	1.58 (3H, s)	14.8	1.58 (3H, s)	16.2
20	1.57 (3H, s)	14.6	1.56 (3H, s)	14.6
1′	–	–	3.38 (3H, s)	56.2

Note: [a]δ(H) are overlapped.

In the $^1H-^1H$ COSY spectrum (Figure 2), a methine proton at δ 0.79–0.90 (1H, m, H-1) was coupled with another methine proton at δ 1.22–1.36 (1H, m, H-2) and methylene protons at δ 1.98–2.15 (2H, m, H-14). H-2 was further coupled with an olefinic proton at δ 6.22 (1H, d, $J = 10.8$ Hz, H-3) and H-14 coupled with methylene protons at δ 1.80–2.30 (2H, m, H-13). Furthermore, a cross peak between H-6 and H-7 could be discerned in the $^1H-^1H$ COSY experiment. The information concerning the location of these units was obtained from the HMBC experiment (Figure 2). The HMBC experiment yielded correlations from H-18 to both C-3 and C-5, H-7 to both C-5 and C-9, and H-19 to C-7. In addition, correlations were discerned between H-11 and C-9, as well as between H-20, H-13, and C-11. The above long-range correlations suggested the presence of a 14-member macrocyclic ring. In addition, correlations were observed between gem-dimethyls H-16 (δ 1.15, 3H, s), H-17 (δ 1.16, 3H, s) and C-1 (δ 38.3) in the HMBC experiment, together with the correlation between two typical cyclopropyl protons at δ 0.79–0.90 (1H, m, H-1) and 1.22–1.36 (1H, m, H-2) in the $^1H-^1H$ COSY spectrum, indicating the presence of a cyclopropyl ring. Thus, compound **1** was considered to be a casbane-type diterpenoid [4].

The relative stereochemistry of **1** was determined by the NOESY experiment (Figure 3). The key NOEs were observed between H-1/H-3, H-3/H-17, H-1/H-17, and H-2/H-16, which gave β-oriented H-1 and α-oriented H-2. In addition, the NOESY experiment showed correlations between H-3/H-6, H-3/H-19, H-7/H-10, H-11/H-1, and H-11/H-13, whereas correlations were not observed between H-3/H-18, H-7/H-19, and H-11/H-20. From these results, **1** was determined to have a 3,7,11 all-*E*-tirene system. Thus, the structure of **1** was confirmed as 18-hydroxy-1β*H*, 2α*H*-casba-3*E*,7*E*,11*E*-trien-5-one, named pekinenin A.

Compound **2** was obtained as a yellow oil and was assigned the molecular formula $C_{21}H_{32}O_3$ by its HR-ESI-MS at *m/z* 331.2258 $[M-H]^-$. Compound **2** exhibited almost identical UV and IR absorptions, ^1H and ^{13}C NMR data to compound **1**. Analysis of the ^1H, ^{13}C NMR, and HMQC spectra (Table 1) yielded a methoxyl at δ 3.38 (H-1′), a

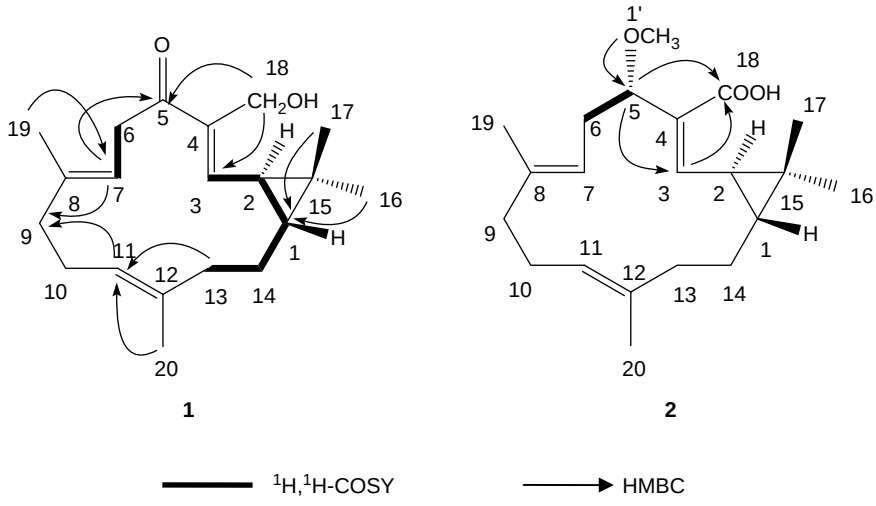

Figure 2. The key HMBC and COSY correlations for compounds **1** and **2**.

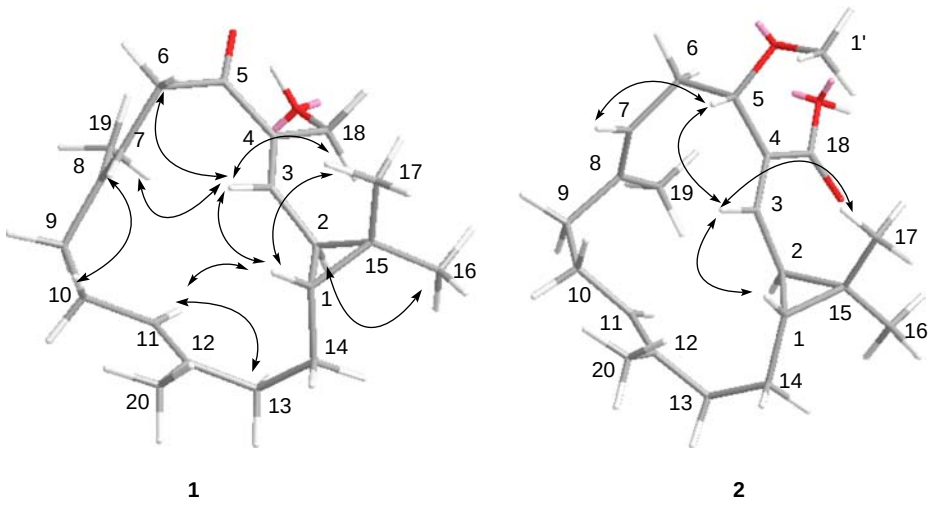

Figure 3. The key NOESY correlations for compounds **1** and **2**.

methine signal at δ 87.1 (C-5, linked to methoxyl group). The up-field shift of carbonyl group from δ 202.5 (C-5) in **1** to δ 167.5 (C-18) in **2** indicated the presence of one carboxyl group. The HMBC (Figure 2) spectrum showed correlations between a methine proton at δ 3.70 (1H, dd, $J = 5.4$, 11.1 Hz, H-5) and both an olefinic carbon at δ 152.9 (C-3) and a carboxyl carbon at δ 167.5 (C-18), and between methoxyl protons at δ 3.38 (3H, s, H-1′) and a methine signal at δ 87.1 (C-5). Furthermore, a cross peak was observed between H-3 and C-18. In the ^{1}H–^{1}H COSY spectrum (Figure 2), a correlation was discerned between H-5 and H-6. Therefore, the carboxyl group was situated in 18th position, and the methoxyl group was confirmed to be linked to C-5. The NOE spectrum (Figure 3) showed signals between H-5/H-3, H-5/H-7, H-3/H-1, and H-3/H-17, which gave the α-oriented methoxyl group. Compared with compound **1**, the structure and relative stereochemistry of the remaining part of compound **2** were identical to those of compound **1**. The structure of **2** was, therefore, elucidated as 5α-methoxy-1β*H*,2α*H*-casba-3*Z*,7*E*,11*E*-trien-18-oic acid, named pekinenin B.

Casbane-type diterpenoids are rare in the plant kingdom and only four compounds have been found so far in *Euphorbia* distributed in China [5].

The cytotoxicity of compounds **1** and **2** was evaluated against Hela, MCF-7, and C6 cancer cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [6]. Compound **1** showed moderate cytotoxic activity against Hela and C6 cancer cell lines with values of 42.97 and 50.00 μM, respectively, and very low activity with an IC_{50} value of 118.76 μM against human MCF-7 cancer cells. Meanwhile, compound **2** had little activity ($IC_{50} > 150$ μM) against all three human cancer cell lines tested.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter. UV spectra were obtained on a Shimadzu UV-2201 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrophotometer. The NMR data were recorded on a Bruker AV-600 (600 MHz for NOESY) and a Varian INOVA-300 (300 MHz for ^{1}H

and 75 MHz for ^{13}C NMR) in $CDCl_3$ with TMS as the internal standard. The HR-ESI-MS data were obtained on a Waters LCT Premier XE time-of-flying mass spectrometer. Chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Group, Co., Qingdao, China), ODS (Octadecylsilyl, 30–50 μm; Tianjin Mical Reagent Co., Tianjin, China), and preparative HPLC (Hitachi-L-7110 pump, Hitachi L-7420 UV spectrophotometric detector at 254 nm, YMC C_{18} reversed-phase column).

3.2 Plant material

The roots of *E. pekinensis* were collected in Yulin City, Guangxi Province of China in August 2008, and was identified by Prof. Jincai Lu (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University). A voucher specimen (No. 20080830) is deposited in the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

Dried roots of *E. pekinensis* (12 kg) were extracted with 72 liters of 95% EtOH ($\times 3$) under reflux conditions for 3 h to give a crude extract, which was suspended in H_2O and extracted with PE, $CHCl_3$, and AcOEt, successively, to yield a PE-soluble fraction (356 g), a $CHCl_3$-soluble fraction (94.5 g), and an AcOEt-soluble fraction (186 g). A part of the PE-soluble fraction (165 g) was subjected to CC (silica gel, gradient of PE–AcOEt 100:1-0:100) to afford fractions 1–24 (108.5 g). Fractions 15–20 (32.0 g) were resubjected to CC (ODS, MeOH–H_2O 80:20) to obtain fraction D (6.3 g). Fraction D was separated by CC (ODS, MeOH–H_2O 60:40, MeOH–H_2O 70:30) to yield fraction D-1 (632.3 mg) and fraction D-2 (874.5 mg), which were further subjected to preparative reversed-phase HPLC (MeOH–H_2O 75:25, flow rate 2.5 ml/min, wavelength 254 nm) to obtain **1** (35 mg) and **2** (55 mg), respectively.

3.3.1 Compound *1*

Yellow oil (35 mg). $[\alpha]_D^{20}$ −58.1 ($c = 1.00$, $CHCl_3$). UV ($CHCl_3$) λ_{max} (nm): 269. IR (KBr) ν_{max} (cm^{-1}): 3417, 2926, 1750, 1670, 1626, 1438, 1380, 1279, and 1113. ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data: see Table 1. HR-ESI-MS *m/z*: 627.4387 $[2M + Na]^+$ (calcd for $C_{40}H_{60}O_4Na$, 627.4389).

3.3.2 Compound *2*

Yellow oil (55 mg). $[\alpha]_D^{20}$ +86.4 ($c = 1.00$, $CHCl_3$). UV ($CHCl_3$) λ_{max} (nm): 249. IR (KBr) ν_{max} (cm^{-1}): 3400, 2932, 1725, 1680, 1628, 1449, 1378, 1275, 1119, and 1074. ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data: see Table 1. HR-ESI-MS *m/z*: 331.2258 $[M - H]^-$ (calcd for $C_{21}H_{31}O_3$, 331.2273).

3.4 Cytotoxicity of compounds 1 and 2

The human cancer cell lines were cultivated in humidified incubators (5% CO_2 and 37°C). The cells were grown in Dulbecco's modified eagle medium containing 10% fetal bovine serum. The cells were seeded in 96-well plates and cultivated for 12 h and then treated with compounds of five concentrations (12.5, 25, 50, 100, 200 μM) for 48 h. Then, 10 μl MTT (5 mg/ml) was added to each well, and cells were incubated for additional 4 h. Then, DMSO (100 μl per well) was added to dissolve the formazan crystals. Absorbance was measured at 490 nm by enzyme immunoassay instrument (Bio-Rad Model 680, Bio-Rad, Hercules, CA, USA). Cytotoxicity was determined as described previously.

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