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PHYTOCHEMICAL CONSTITUENTS ISOLATED FROM *Euphorbia rapulum*

Xiao Xue Liu, Hongmei Ma, Wenjing He, Yun Sun, and Wei Lan*

Phytochemical studies on the roots of Euphorbia rapulum *afforded a new casbane diterpenoid, 8,12-dihydroxy-1*βH*,2*αH*-casba-3*E*,7*E*,11*E*-trien-5-one (**1**), together with four other known compounds, helioscopinolide A (**2**), 19-hydroxyjolkinolide E (**3**), jolkinolide E (**4**), and euphopilolide (**5**). Their structures were established on the basis of spectroscopic analysis and chemical evidence. All the isolated compounds were evaluated for cytotoxic activities against HepG2, MCF-7, and C6 cell lines, while all compounds showed weak selective activity against all three cell lines.*

Keywords: *Euphorbia rapulum*, diterpenoids, cytotoxic activities.

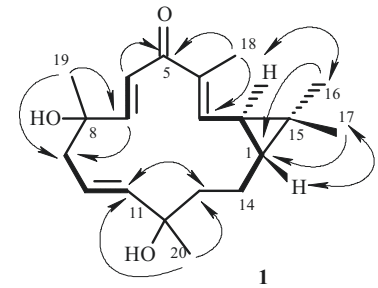
The genus *Euphorbia*, comprising more than 2000 species, belongs to the family Euphorbiaceae. It is characterized by a toxic and highly skin-irritating latex [1]. *Euphorbia* has high pharmacological potential, and parts of this genus are used to treat different diseases such as skin disease, gonorrhea, and warts [2, 3]. *E. rapulum*, a perennial herb of the *Euphorbia* genus, is primarily distributed in Xinjiang and Central Asia. As far as our literature survey could confirm, only a few studies on *E. rapulum* have been reported [4]. Therefore, the aim of our study was to evaluate its biologically active compounds. Methanol extracts of *E. rapulum* were investigated, and we obtained a new casbane diterpenoid, 8,12-dihydroxy-1*βH*,2*αH*-casba-3*E*,7*E*,11*E*-trien-5-one (**1**), together with other four known compounds, helioscopinolide A (**2**), 19-hydroxyjolkinolide E (**3**), jolkinolide E (**4**), and euphopilolide (**5**). The present paper describes the isolation, structure elucidation, and cytotoxic activity of these compounds.

Compound **1** was isolated as a yellow oil. HR-ESI-MS analysis exhibited a molecular ion peak at *m/z* 319.2246 $[M + H]^+$, corresponding to a molecular formula of $C_{20}H_{30}O_3$ and implying six degrees of unsaturation. The IR spectrum exhibited bands for hydroxyl and olefinic substituents at 3409 cm^{-1} and 1650 cm^{-1}, respectively. These observations were in agreement with the signals in the ^{13}C NMR spectrum for two secondary oxygenated carbons (δ 71.9 and 74.0), a trisubstituted double bond (δ 150.6 and 135.2), and a ketocarbonyl group (δ 200.4), indicating the presence of an *α,β*-unsaturated carbonyl group. The ^1H NMR spectrum (Table 1) demonstrated resonances of five methyl singlets at δ 1.85 (s, H_3-18), 1.41 (s, H_3-19), 1.37 (s, H_3-20), 1.17 (s, H_3-16), and 1.08 (s, H_3-17) and five olefinic protons at δ 6.03 (1H, d, J = 10.4 Hz, H-3), 6.05 (1H, d, J = 16.2 Hz, H-6), 6.51 (1H, d, J = 16.2 Hz, H-7), 6.01 (1H, dd, J = 10.8, 15.5 Hz, H-10), and 5.87 (1H, d, J = 15.5 Hz, H-11). Analysis of the ^{13}C NMR spectrum displayed 20 carbon resonances, which were classified by DEPT spectra (Table 1) as six olefinic carbons [δ, 150.6 (C-3), 135.2 (C-4), 127.3 (C-6), 152.1 (C-7), 121.8 (C-10), 141.5 (C-11)] and two hydroxyl carbons [δ 71.9 (C-8) and 74.0 (C-12)]. A detailed analysis of the 2D NMR spectra of **1** allowed the assignment of all proton and carbon atoms. On the basis of ^1H–^1H COSY corrections, the three structural fragments **a** (–C-3–C-2–C-1–C-14–), **b** (–C-9–C-10–C-11–), and **c** (–C-6–C-7–) (Fig. 1) were established. Since compound **1** has three π-bonds, **1** must be bicyclic to satisfy the six degrees of unsaturation requirement. Besides, the presence of both a quaternary carbon (δ 29.3, C-15) and a gem-dimethyl functionality [δ_3 1.17 (s, H_3-16), 1.08 (s, H_3-17)], together with the HMBC correlation of H_3-16/C-1, 2, 15, 17 and H_3-17/C-1, 2, 15, 16, indicated a substituted cyclopropyl ring.

School of Traditional Chinese Medicine, XinJiang Medical University, 832000, Urumqi, P. R. China, e-mail: m13364798224@163.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 5, September–October, 2018, pp. 772–774. Original article submitted June 13, 2017.

TABLE 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_C	δ_H	HMBC	C atom	δ_C	δ_H	HMBC
1	39.3	1.13 (1H, m)	–	12	74.0	–	–
2	31.9	1.19 (1H, m)	–	13	44.2	1.85 (1H, m)	11, 12, 14, 20
3	150.6	6.03 (1H, d, J = 10.4)	1, 5, 18			1.71 (1H, m)	11, 12, 14, 20
4	135.2	–	–	14	24.1	1.97 (1H, m)	1, 12
5	200.4	–	–			1.29 (1H, m)	–
6	127.3	6.05 (1H, d, J = 16.2)	5, 8	15	29.3	–	–
7	152.1	6.51 (1H, d, J = 16.2)	9, 19	16	21.5	1.17 (3H, s)	1, 2, 15, 17
8	71.9	–	–	17	24.0	1.08 (3H, s)	1, 2, 15, 16
9	46.2	2.23 (1H, dd, J = 10.8, 14.5)	10, 11	18	12.4	1.85(3H, s)	3, 5
		2.54 (1H, br.d, J = 14.5)	7, 8, 10,11	19	26.2	1.41 (3H, s)	7, 8, 9
10	121.8	6.01 (1H, dd, J = 10.8, 15.5)	–	20	28.6	1.37 (3H, s)	11, 12, 13
11	141.5	5.87 (1H, d, J = 15.5)	9, 10				



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COSY ⟶ HMBC ⟷ NOESY

Fig. 1. ^{1}H–^{1}H COSY, HMBC and NOESY correlations of compound **1**.

Thus, the remaining ring was deduced to be a 14-membered macrocycle, which could be confirmed on the basis of HMBC correlations. The three fragments a–c could be fully connected by inserting quaternary carbons C-4, C-5, C-8, and C-12. At this point, the planar structure of **1** was completed, consistent with a casbane–type diterpenoid [5].

The relative configuration of **1** was established on the basis of the NOESY and ^{13}C NMR experiments (Fig. 1). The *E* geometry of the double bond $\Delta^{3(4)}$ was deduced by the δ_C values of CH_3-18 (< 20 ppm) [6]. Furthermore, the $\Delta^{6(7)}$, $\Delta^{10(11)}$ was implied as *E* owing to the coupling constants (J_{6-7} = 16.2 Hz and J_{10-11} = 15.5 Hz). There are four chiral centers (C-1, C-2, C-8, and C-12) in the molecule. The correlations between H-1/H_3-17, H-1/H-3, and H-2/H_3-16, indicated that the junction of the cyclopropyl ring was *trans*. This assignment was confirmed by the diagnostic ^{13}C NMR chemical shifts of the germinal methyls C-16 and C-17 (δ 21.5 and 24.0), which were significantly different in comparison with those of the co-occurring model compound sinularcasbane K (δ_C 16.0 and 29.1) [7]. Because of the flexible nature of the 14-membered macrocylic ring, the configuration of C-8 and C-12 could not be decided by the NOESY experiment. Thus, the difference between the structures of **1** and sinularcasbane K was at the configuration of the junction centers (C-1 and C-2). Finally, the structure of compound **1** was elucidated to be 8,12-dihydroxy-1βH,2αH-casba-3*E*,7*E*,11*E*-trien-5-one.

The known compounds were identified as helioscopinolide A (**2**), 19-hydroxyjolkinolide E (**3**), jolkinolide E (**4**), and euphopilolide (**5**) by comparison with the literature data.

The cytotoxicity of all isolated compounds were evaluated against HepG2, MCF-7, and C6 cancer cell lines by the MTT assay. All compounds showed weak inhibitory activity against all three cell lines [8].

EXPERIMENTAL

General Experimental Procedures. UV spectra were obtained on a Shimadzu UV-2201 spectrophotometer (Shimadzu Co., Tokyo, Japan). IR spectra were recorded on a PerkinElmer 577 spectrophotometer (PerkinElmer Co., Massachusetts, USA). NMR spectra were measured with a Bruker Avance III (HD-400 and 600 MHz) spectrometer in $CDCl_3$

with TMS as internal standard (Bruker, Karlsruhe, Germany). The HR-ESI-MS data were obtained on a Waters LCT Premier XE time-of-flight mass spectrometer (Waters Co., Shanghai, China). Chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Group, Co., Qingdao, China) and ODS (Octadecylsilyl, 30–50 mm; Tianjin Mical Reagent Co., Tianjin, China), followed by preparative HPLC (Hitachi-L-7110 pump, Hitachi L-7420 UV spectrophotometric detector at 210 nm, YMC C_{18} reversed-phase column).

Plant Material. Whole plants of *Euphorbia rapulum* were collected from Ili Kazakh Autonomous Prefecture, Xinjiang Uighur Autonomous Region, China in May 2012. Plants were identified by Dr. Yong Tan (School of Pharmacy, Shihezi University). A voucher specimen (2012050312) has been deposited at the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

Extraction and Isolation. Dried plants of *E. rupulum* (2.0 kg) were extracted with 95% EtOH three times (20 L, 16 L, 16 L) under reflux conditions, each for 2 h, to yield a crude extract (200.0 g) under reduced pressure. The residue was subjected to silica gel column chromatography (gradient of PE–acetone; 100:1–0:100) to afford fractions 1–8. Fraction 5 (15.0 g) was separated using column chromatography (CC) (ODS, MeOH–H_2O; 35–75%) to obtain fractions F1–F5. Purification of fraction F2 (450.0 mg) using CC (ODS, MeOH–H_2O 45%), followed by HPLC (CH_3CN–H_2O, 65:35, flow rate 2.2 mL/min, wavelength 210 nm), yielded **2** (7.5 mg, t_R 40 min) and **3** (10.0 mg, t_R 47 min). Fraction 8 (29.1 g) was separated using column chromatography (CC) (ODS, MeOH–H_2O; 30–80%) to obtain fractions E1–E6. Purification of fraction E2 (300.0 mg) using CC (ODS, MeOH–H_2O 40%), followed by HPLC (CH_3OH–H_2O, 70:30, flow rate 2.0 mL/min, wavelength 210 nm), yielded **1** (4.0 mg, t_R 35min). Fraction 1 (3.5 g) was recrystallized to yield **4** (100.0 mg). Fraction 2 (400.0 mg) was recrystallized to yield **5** (15.0 mg).

8,12-Dihydroxy-1*βH*,2*αH*-casba-3*E*,7*E*,11*E*-trien-5-one (1), $C_{20}H_{30}O_3$, yellow oil. UV ($CHCl_3$, λ_{max}, nm): 284 (5.53). IR (KBr, ν_{max}, cm^{-1}): 3434, 2921, 1628, 1454, 1384, 1276, 1114. For ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data, see Table 1. HR-ESI-MS *m/z* 319.2246 $[M + H]^+$ (calcd for $C_{20}H_{31}O_3$, 319.2251).

Helioscopinolide A (2), $C_{20}H_{28}O_3$, white crystals. ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data are as in [9].

19-Hydroxyjolkinolide E (3), $C_{20}H_{28}O_3$, white crystals. ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data are as in [10].

Jolkinolide E (4), $C_{20}H_{28}O_2$, colorless needle crystals. ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data are as in [11].

Euphopilolide (5), $C_{20}H_{28}O_3$, colorless needle crystals. ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data are as in [12].

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