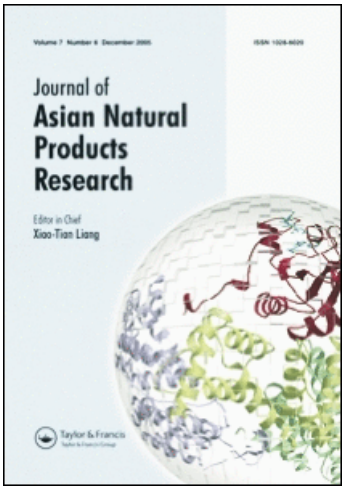

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Guang-Miao Fu[a]; Hai-Lin Qin[a]; Shi-Shan Yu[a]; Bo-Yang Yu[b]
[a] Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China [b] College of Chinese Materia Medica, China Pharmaceutical University, Nanjing, China

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Yuexiandajisu D, a novel 18-nor-rosane-type dimeric diterpenoid from *Euphorbia ebracteolata* Hayata

GUANG-MIAO FU†‡, HAI-LIN QIN‡, SHI-SHAN YU‡ and BO-YANG YU†*

†College of Chinese Materia Medica, China Pharmaceutical University, Nanjing 210009, China
‡Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. No.1 Xian Nong Tan Street, Beijing 100050, China

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Yuexiandajisu D, a novel 18-nor-rosane-type dimeric diterpenoid, was isolated from the roots of *Euphorbia ebracteolata* Hayata. Its structure was elucidated as 6,3′:7,2′-diepoxy-di-2,3-dihydroxy-18-nor-ros-1(10),2,4,15-tetraene by spectroscopic techniques (HSQC, HMBC, DQF-COSY, TOCSY, NOESY) and chemical methods. Yuexiandajisu D showed moderate cytotoxic activity against cancer cell lines on HCT-8 and Bel-7402, with IC_{50} values of 2.66 and 3.76 μM, respectively.

Keywords: Euphorbiaceae; *Euphorbia ebracteolata*; Yuexiandajisu D; 18-Nor-rosane diterpenoid; Dimeric diterpenoid

1. Introduction

Euphorbia ebracteolata Hayata (Euphorbiaceae), distributed in China, Korea and Japan sporadically, is a perennial herbaceous plant. The roots of this plant, named "LangDu" which was classified as a toxic drug and must be used with great care for its high potency and relatively violent pharmacological effects, has been used for the treatment of oedema, indigestion, cough, asthma and chronic bronchitis in traditional Chinese medicine for more than two thousand years [1]. Steroids [2], diterpenes including two casbane-type diterpenoids and an isopimarane diterpenoid [3,4], triterpenes [5], flavonol glycosides [6], tannins [7] and phloroglucinol derivatives [8,9] have been reported from *E. ebracteolata.* For studies on the relationship between constituents and the bioactivities on Chinese medicinal plants, we re-investigated the constituents of the title plant. In our previous study, we reported two new phloroglucinol derivatives from the $CHCl_3$ soluble fraction of the ethanol extracts of the roots of *E. ebracteolata*. Our continuing investigation of this fraction led to the isolation of yuexiandajisu D (**1**), which possesses an uncommon 18-nor-rosane-type dimeric diterpenoid. Compound **1** showed activity against the growth of cancer cell lines HCT-8 and Bel-7402

*Corresponding author. E-mail: boyangyu@cpu.edu.cn

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with the IC_{50} values at 2.66 and 3.76 μM, respectively. This paper describes the structure elucidation and bioassay results of **1**.

2. Results and discussion

Compound **1** was obtained as light yellow amorphous powder. The molecular formula, $C_{38}H_{48}O_4$, consistent with 15 degrees of unsaturation, was determined by HRFAB-MS at *m/z* 569.3612 $[M + H]^+$ and HREI-MS at *m/z* 568.3564 $[M]^+$. The IR spectrum of **1** showed absorptions of hydroxyl (3448 cm^{-1}) and phenyl (1605, 1480 cm^{-1}) groups. The ^1H NMR spectrum of **1** displayed characteristic signals for two aromatic protons at δ_H 6.75 (1H, s, H-1) and 6.74 (1H, s, H-1′), six olefinic protons at δ_H 5.87 (1H, dd, $J = 17.5$, 11.0 Hz, H-15), 5.06 (1H, dd, $J = 17.5$, 1.5 Hz, H-16a), 4.90 (1H, dd, $J = 11.0$, 1.5 Hz, H-16b) and 5.90 (1H, dd, $J = 17.5$, 10.5 Hz, H-15′), 5.02 (1H, dd, $J = 17.5$, 1.0 Hz, H-16′a), 4.88 (1H, dd, $J = 10.5$, 1.0 Hz, H-16′b), two oxygenated methine protons at δ_H 4.96 (1H, d, $J = 3.5$ Hz, H-6) and 4.42 (1H, dd, $J = 12.0$, 3.5 Hz, H-7), and six tertiary methyl signals at δ_H 2.37 (3H, s, H-19), 2.07 (3H, s, H-19′), 1.12 (3H, s, H-20), 0.91 (3H, s, H-17), 1.04 (6H, s, H-17′, 20′). The ^{13}C NMR and DEPT spectral data indicated that **1** contains 38 carbons, including six tertiary methyls, ten methylenes (two olefinic), eight methines (two oxygenated and four olefinic) and 14 quaternary carbons (ten olefinic). All of these are similar to neither typical casbane-type diterpenes nor typical isopimarane-type diterpenes found in *E. ebracteolata*, but are suggestive that **1** would appear to be an asymmetrical dimeric diterpenoid. Compound **1** showed fragmentation ion at *m/z* 284 $[M - 284]^+$ in the EI-MS spectrum, which indicated **1** contains one aromatic ring and one substituted double bond in each diterpene moiety of the molecule. By careful analysis of the DQF-COSY, HSQC and HMBC spectra data, it was established that **1** possessed a modified 18-nor-rosane-type dimeric carbon skeleton.

In the HMBC spectrum of **1,** the long-range correlation between H-6 (δ 4.96) and C-3′ (δ 139.1) indicated C-3′ was connected to C-6 by an ether linkage. Similarly, the cross-peak between H-7 (δ 4.42) and C-2′ (δ 140.0) indicated that C-2′ was connected to C-7 by an ether linkage. In the NOESY spectrum (figure 1) of **1**, correlations between δ 1.12 (CH_3-20) and 4.42 (H-7) indicating both CH_3-20 and H-7 adopt a β-orientation; accordingly, H-6, H-8 and CH_3-17 are α-oriented. Similarly, CH_3-20′ is β-oriented while H-8′ and CH_3-17′ are α-oriented. Detailed assignments of the protons and carbons were accomplished by means of HSQC, HMBC (figure 2), DQF-COSY, NOESY and TOCSY experiments. On the basis of the above evidence, the structure of **1** was established as 6,3′:7,2′-diepoxy-di-2,3-dihydroxy-18-nor-ros-1(10),2,4,15-tetraene.

18-nor-rosane-type dimeric diterpenoid was isolated from the *Euphorbia* genus for the first time. The cytotoxicity of **1** was evaluated against several human cancer cell lines as shown in table 2. Adriamycin was used as a positive control.

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XT-4 micromelting point apparatus and are uncorrected. Optical rotations were determined on a Perkin–Elmer 241 automatic digital polarimeter in

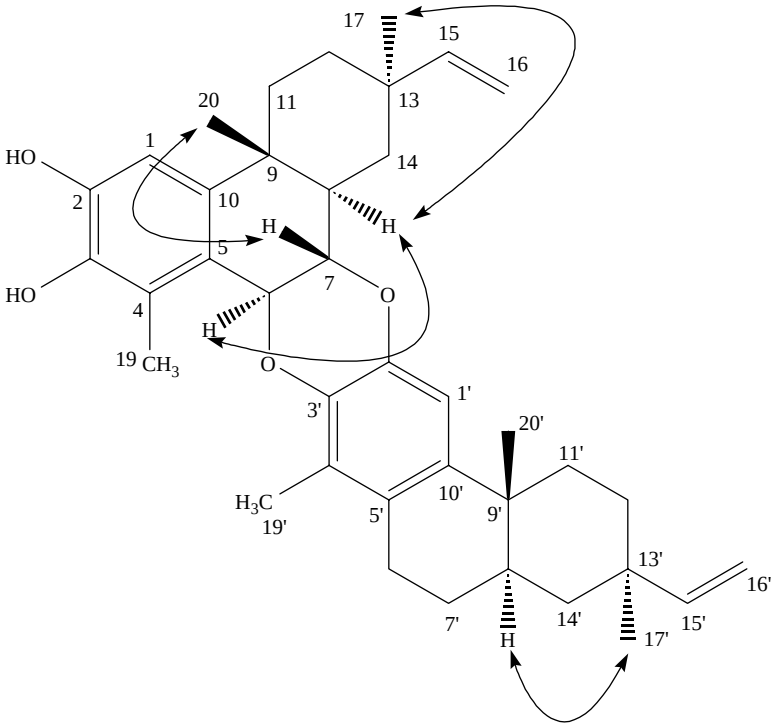

Figure 1. Structure and key NOESY correlations of compound **1**.

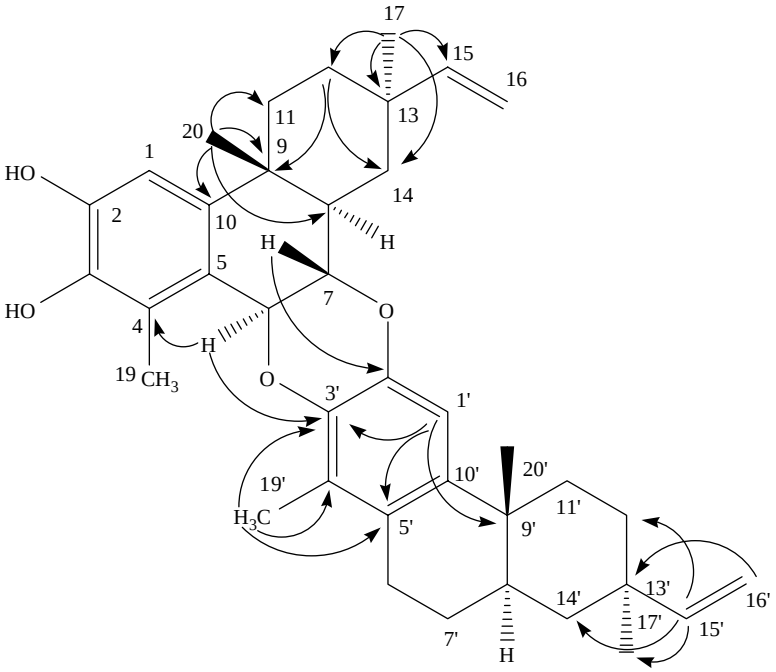

Figure 2. Key HMBC correlations of compound **1**.

$CHCl_3$ at 25°C. IR spectra were recorded using a Nicolet-Impact 400 IR spectrometer with KBr disks. UV spectra were obtained on a Shimadzu UV-260 spectrometer. ^1H NMR, ^{13}C NMR, DEPT, COSY, HMQC and HMBC experiments were performed on an Inova 500 FT NMR spectrometer. TMS was used as internal standard. HRESI-MS and HRFAB-MS were measured on an AutoSpec Ultima-TOF spectrometer. Silica gel GF_{254} for TLC and silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company, Qingdao, China. Solvents were analytical grade and purchased from Beijing Chemical Company, Beijing, China.

3.2 Plant material

The roots of *Euphorbia ebracteolata* were purchased from Beijing Chinese Medicinal Herbs Corporation in October 2000 and identified by Dr. Bo-yang Yu, professor of China Pharmaceutical University, and by comparison with the authentic samples in the Herbarium of China Pharmaceutical University. A voucher specimen (Herbarium No. 20004003) of the plant is deposited at the Herbarium of China Pharmaceutical University, Nanjing, Jiangsu, China.

3.3 Extraction and isolation

The air-dried roots (4.75 kg) of *E. ebracteolata* were ground and refluxed three times with 95% EtOH (40 L). The 95% EtOH solution was combined and evaporated *in vacuo* to yield 320 g of residue. The residue was suspended in water and extracted successively with petroleum, $CHCl_3$ and n-BuOH. The $CHCl_3$ extract (158 g) was obtained, part of which (86 g) was chromatographed on a silica gel (1000 g) column (7 × 140 cm) eluting with $CHCl_3$/MeOH (10:1, 8:2, 7:3, 6:4, 5:5, 4:8, 2:8, 0:1, 5000 ml each eluent) to obtain 80 fractions (500 ml each). Five fractions (I, 13.2 g; II, 8.6 g; III, 12.6 g; IV, 4.2 g; V, 15.6 g) were obtained according to the differences in composition indicated by TLC analysis. Fraction I was resubmitted to silica gel (300 g) column (4 × 110 cm) chromatography eluting with $CHCl_3$/MeOH (20:1, 10:1, 8:2, 7:3, 6:4, 5:5, 500 ml each eluent) to obtain six subfractions (A, 1.8 g; B, 2.2 g; C, 3.6 g; D, 1.8 g; E, 1.4 g; F, 0.9 g). Fraction B was subjected to Sephadex LH-20 (150 g) column (3 × 140 cm) chromatography eluted with $CHCl_3$/MeOH (2:1) to yield compound **1** (62 mg).

3.3.1 Yuexiandajisu D (1). Light yellow amorphous powder; mp 157–158 °C; $[\alpha]_D^{25}$ + 68.6 (*c* 0.70, $CHCl_3$); IR (KBr) ν_{max} (cm^{-1}): 3448, 3436, 2967, 2927, 1605, 1480, 1453, 1299, 1284, 1214, 1094, 909, 854; UV (MeOH) λ_{max} (log ε) (nm): 286 (1.004); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) NMR data are shown in table 1; ESI-MS *m/z*: 569 $[M + H]^+$; EI-MS *m/z*: 568 M^+(80), 286 (70), 284 (95), 271 (100), 269 (60), 227 (25), 213 (44), 201 (53), 187 (37), 163 (25), 95 (48); HREI-MS *m/z*: 568.3564 M^+ (calcd for $C_{38}H_{49}O_4$, 568.3553); HRFAB-MS *m/z*: 569.3612 $[M + H]^+$ (calcd for $C_{38}H_{49}O_4$, 569.3631).

3.4 Cytotoxicity assay

We evaluated the cytotoxic activity of compound **1** on KB, HCT-8, Bel-7402, BGC-823 and A549 cell lines (see table 2). Adriamycin was used as a positive control. After the cells were

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of the compound **1**[a] (in $CDCl_3$ δ in ppm, J in Hz).

Position	*δ (H)*	*δ (C)*	*Position*	*δ (H)*	*δ (C)*
1	6.75 (1H, s)	109.5	1′	6.74 (1H, s, 1′-H)	108.9
2		144.3	2′		140.0
3		141.0	3′		139.1
4		125.6	4′		124.6
5		123.8	5′		126.4
6	4.96 (1H, d, J = 3.5 Hz)	69.4	6′	2.60 (1H, dd, J = 17.0, 7.0 Hz)	27.0
				2.69 (1H, m)	
7	4.42 (1H, dd, J = 12.0, 3.5 Hz)	73.1	7′	1.67 (1H, m)	26.0
				1.57 (1H, m)	
8	2.40 (1H, m)	36.4	8′	1.78 (1H, br t, J = 12.5 Hz)	36.6
9		39.0	9′		36.8
10		140.9	10′		141.8
11	1.97 (1H, m)	34.7	11′	2.03 (1H, br d, J = 12.5 Hz)	34.4
	1.68 (1H, m)			1.62 (1H, m)	
12	1.40 (2H, m)	32.6	12′	1.42 (2H, m)	33.2
13		36.1	13′		36.7
14	1.70 (2H, m)	32.6	14′	1.47 (1H, m)	40.0
				1.22 (1H, br d, J = 13.5 Hz)	
15	5.87 (1H, dd, J = 17.5, 11.0 Hz)	151.5	15′	5.90 (1H, dd, J = 17.5, 10.5 Hz)	151.1
16	5.06 (1H, dd, J = 11.0, 1.5 Hz)	109.2	16′	5.02 (1H, dd, J = 10.5, 1.0 Hz)	108.8
	4.90 (1H, dd, J = 17.5, 1.5 Hz)			5.88 (1H, dd, J = 17.5, 1.0 Hz)	
17	0.91 (3H, s)	23.4	17′	1.04 (3H, s)	23.1
19	2.37 (3H, s)	11.4	19′	2.07 (3H, s)	11.2
20	1.12 (3H, s)	22.4	20′	1.04 (3H, s)	21.6

[a] Assignments were aided by 2D NMR COSY, HSQC and HMBC experiments.

Table 2. Cytotoxic activity of **1**[a].

	Cell lines				
Compound	*HCT-8*	*KB*	*A549*	*Bel-7402*	*BGC-823*
1	2.66	>10.00	>10.00	3.76	>10.00
Adriamycin	0.21	0.42	0.67	0.48	0.38

[a] Values are means of three experiments and results are expressed as IC_{50} values in μM.

continuously treated with the samples for 72 h, the supernatant was doffed off and 0.1 ml MTT (0.5 mg/ml in RPM1640) was added after each well had been carefully washed with RPM1640. The cell growth was measured with an MTT assay procedure, and the IC_{50} values calculated from a dose-dependent curve on KB, HCT-8, Bel-7402, BGC-823 and A549 cell lines.

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References

[1] Jiangsu College of New Medicine, *Dictionary of Chinese Materia Medica*, Vol. 2, pp. 1898–1900, Shanghai Science and Technology Press, Shanghai (1986).
[2] H.Q. Zhang, Y.M. Ding, G.M. Chen, Y.F. Dong, Y.L. Zhu. *Acta Bot. Sin.*, **29**, 429 (1987).
[3] Z.H. Xu, J. Sun, R.S. Xu, G.W. Qin. *Phytochemistry*, **49**, 149 (1998).
[4] Z.H. Xu, G.W. Qin, R.S. Xu. *J. Asian Nat. Prod. Res.*, **2**, 257 (2000).
[5] X.F. Sun, S.P. Wang, Z.R. Zheng. *China J. Chin. Mater. Med.*, **24**, 226 (1999).
[6] B.T. Ahn, K.J. Oh, J.S. Ro, K.S. Lee. *Planta Med.*, **62**, 383 (1996).
[7] B.T. Ahn, S.C. Lee, W.Y. Park, S.H. Lee, J.S. Ro, K.S. Lee, E.G. Ryu. *Kor. J. Phamacogn.*, **23**, 211 (1992).
[8] Y.F. Dong, Y.M. Ding. *J. Plant Res. Environ.*, **1**, 1 (1992).
[9] H.Q. Zhang, Y.M. Ding. *J. Plant Res. Environ.*, **1**, 6 (1992).