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A NEW LATHYRANE DITERPENOID ESTER FROM *Euphorbia dracunculoides*

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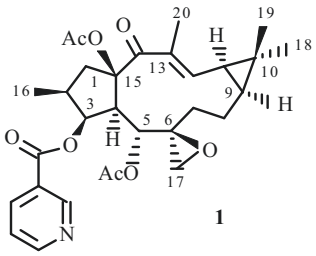
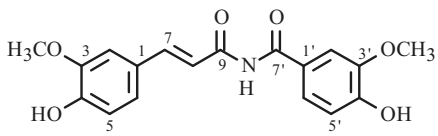
*A new lathyrane diterpenoid ester, euphordracunculin C (**1**), together with a phenolic amide, 4-hydroxy-*N*-[3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propen-1-yl]-3-methoxybenzamide (**2**), was isolated from the aerial parts of* Euphorbia dracunculoides. *The structure of the new compound was elucidated on the basis of spectroscopic analysis including 1D and 2D NMR techniques.*

Keywords: *Euphorbia dracunculoides*, lathyrane, diterpenoid ester.

The genus *Euphorbia* is the largest in the family Euphorbiaceae, comprising more than 2000 species, many of which have been used in traditional Chinese medicine for the treatment of skin diseases, edemas, gonorrhea, migraine, and intestinal parasites and as wart cures [1]. *Euphorbia dracunculoides* Lam., belonging to the genus *Euphorbia*, is a perennial herb distributed in riverbanks, valleys, and roadsides of sandy areas in North Africa, South Europe, and Southwest Asia [2]. It has been used in the folk medicine of India as a laxative and diuretic for many years [3]. However, phytochemical investigations on its diterpenoids are lacking, and only one diterpenoid, named euphorbol, was reported in 1966 [4]. In previous papers we reported the isolation and characterization of two myrinsol diterpenoids [5]. Aiming to searching for more biologically active diterpenoids, we have investigated the aerial parts of *E. dracunculoides*, collected in Xishuang Banna Prefecture, Yunnan Province, People′s Republic of China. As a result, a new lathyrane diterpene ester, euphordracunculin C (**1**), together with a phenolic amide, 4-hydroxy-*N*-[3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propen-1-yl]-3-methoxybenzamide (**2**), was obtained. We present herein the isolation and structure elucidation of this new compound.



1



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Euphordracunculin C (**1**) was obtained as a colorless gum from $CHCl_3$. The HR-ESI-MS data (*m/z* 562.2415 $[M + Na]^+$, calcd 562.2417) of **1** showed the molecular formula $C_{30}H_{37}O_8N$, corresponding to 13 degrees of unsaturation. The IR spectrum of **1** displayed absorptions of carbonyls (1741 cm^{-1}), conjugated double bonds (1624 cm^{-1}), and C–O groups (1026 cm^{-1}).

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

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1α	3.51 (1H, dd, J = 14.4, 8.4)	48.3 (t)	15	–	92.2 (s)
1β	1.62 (1H, overlapped)		16	0.91 (3H, d, J = 6.7)	14.3 (q)
2	2.26 (1H, m)	38.2 (d)	17a	2.51 (1H, d, J = 3.3)	55.5 (t)
3	5.75 (1H, t, J = 2.9)	81.8 (d)	17b	2.35 (1H, br.s)	
4	2.01 (1H, dd, J = 9.0, 2.9)	50.2 (d)	18	1.21 (3H, s)	29.1 (q)
5	6.32 (1H, d, J = 9.0)	65.2 (d)	19	1.22 (3H, s)	16.9 (q)
6	–	59.1 (s)	20	1.88 (3H, s)	12.6 (q)
7a	2.08 (1H, m)	33.6 (t)	3-*O*-Nicotinoyl	–	164.7 (s)
7b	0.92 (1H, overlapped)		1′	–	125.9 (s)
8a	2.02 (1H, m)	20.2 (t)	2′	8.26 (1H, d, J = 7.9)	137.2 (d)
8b	1.72 (1H, m)		3′	7.41 (1H, dd, J = 7.9, 4.7)	123.5 (d)
9	1.09 (1H, m)	35.0 (d)	4′	8.80 (1H, dd, J = 4.7, 1.3)	153.9 (d)
10	–	25.9 (s)	6′	9.20 (1H, s)	151.2 (d)
11	1.50 (1H, dd, J = 11.0, 7.5)	29.3 (d)	5-OAc	–	170.4 (s)
12	6.65 (1H, d, J = 11.0)	144.0 (d)		1.85 (3H, s)	20.9 (q)
13	–	136.2 (s)	15-OAc	–	169.7 (s)
14	–	196.7 (s)		2.25 (3H, s)	22.1 (q)

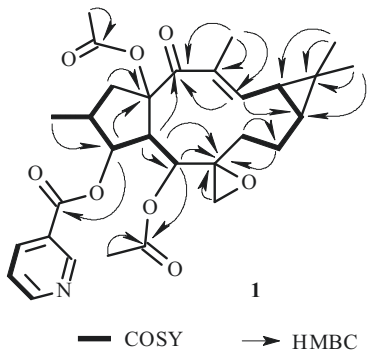

Fig. 1. Key COSY and HMBC correlations of **1**.

The ^{1}H and ^{13}C NMR spectrum of **1** (Table 1) suggested the presence of two acetyl groups [δ_H 2.25 and 1.85; δ_C 170.4, 169.7, 20.9, and 22.1]. The ^{1}H NMR signals at δ 9.20, 8.80, 8.26, and 7.41 as well as the HMBC cross peaks between these protons and carbonyl carbon (δ_C 164.7) revealed the occurrence of nicotinyl ester. Additionally, three methyls (δ 0.91 d, 1.21 s, and 1.22 s), one methyl group at a double bond (δ 1.88 s), and one olefinic proton (δ 6.65 d) were also evident from the ^{1}H NMR spectrum. An α,β-unsaturated carbonyl (δ_C 196.7 s, 144.0 d, and 136.2 s) was observed in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum showed 30 carbon resonances, including the above ten carbons of the substituents (two acetoxy and one nicotinoyloxy groups). The remaining 20 carbons in the ^{13}C NMR experiment, covering four methyls, four methylenes, seven methines, and five quarternary ones, form the characteristic 6,17-epoxylathyrol diterpene skeleton for **1** based on the reported 6,17-epoxylathyrol diterpenes from the genus *Euphorbia* [6–9]. Aiming to confirm the 6,17-epoxylathyrol skeleton and determine the positions of the substituents, we measured the 2D NMR (COSY, HSQC, and HMBC) spectra. By analysis of the 1D and 2D NMR spectra, we define the 6,17-epoxylathyrol skeleton (Fig. 1), in which the double bond was assigned to C-12 and C-13, and an acetyl and a nicotinoyloxy groups were attributed to C-5 and C-3, respectively, by the correlations of H-12 (δ 6.65) with C-11 (δ 29.3), C-9 (δ 35.0), and C-14 (δ 196.7), of H-5 (δ 6.32) with carbonyl (δ 170.4), and of H-3 (δ 5.75) with carbonyl (δ 164.7) in the HMBC spectrum. The 6,17-epoxy ring was determined by the correlations from H-4 (δ 2.01), H-5 (δ 6.32), H-8 (δ 1.72), and H-17 (δ 2.51 and 2.35) to C-6 (δ 59.1). Considering the ^{13}C NMR spectroscopic data and similar 6,17-epoxylathyrol diterpenes in the literatures [6, 7], we see that another acetyl group could only be located at C-15. All the protons and carbons were assigned unambiguously based on further examination of the NMR spectra (Fig. 1). Therefore, the planar structure of **1** was established as 5,15-diacetoxy-3-nicotinoyloxy-6,17-epoxylathyra-12-en-14-one.

The relative configuration of **1** was elucidated by a ROESY experiment. For the reported lathyrane diterpenes, the five-membered ring and the macro-ring forming the skeleton are *trans*-fused, H-4 is α-oriented, and the H_3-16 and C-15 acyloxy group are β-oriented [6–9]. The ROESY correlations observed for H-2/H-4, H-3/H-4, H-4/H_2-17, H-5/H-12, H-9/H-11, H-9/H_3-18, H-11/H_3-18, H-11/H_3-20, and H-12/H_3-19 suggested that H-3, H-9, H-11, and the C-5 acyloxy group were α-oriented, and the double bond was of the *E*-configuration. Consequently, compound **1** was elucidated as 5α,15β-diacetoxy-3β-nicotinoyloxy-6,17-epoxylathyra-12-en-14-one.

EXPERIMENTAL

General. Optical rotations were recorded in MeOH using a JASCO P-1020 polarimeter. UV spectra were acquired in MeOH with a Shimadzu UV-2401PC UV-VIS spectrophotometer. IR spectra were measured on a Bruker Tensor 27 FTIR spectrometer with KBr disks. NMR spectra were recorded in $CDCl_3$ or MeOD using a Bruker Avance III-600 spectrometer, and TMS was used as internal standard. ESI-MS spectra were recorded using a Waters Xevo TQ-S ultrahigh pressure liquid chromatography triple quadrupole mass spectrometer. HR-ESI-MS data were obtained using an Agilent G6230 Q-TOF mass instrument. Semipreparative HPLC was conducted on a Hitachi Chromaster system equipped with a DAD detector and a YMC-Triart C_{18} column (250 mm × 10 mm i.d., 5 μm).

Plant Material. The aerial parts of *Euphorbia dracunculoides* Lam. were collected in September 2012 from Xishuang Banna Prefecture, Yunnan Province, People′s Republic of China, and authenticated by Shun-Cheng Zhang, Xishuang Banna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No. Zhang20120927) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered aerial parts of *E. dracunculoides* (4.0 kg) were extracted with 70% aqueous acetone (10 L × 2 d × 3) at room temperature. The extracts were concentrated to remove organic solvent. The aqueous residue was then partitioned with petroleum ether, EtOAc, and *n*-BuOH sequentially. The petroleum ether-soluble part (48.0 g) was subjected to silica gel column chromatography (CC), eluting with petroleum ether–acetone (1:0–0:1) to afford six fractions (A–F). Fraction E (3.1 g) was firstly separated by MPLC (MCI gel CHP 20P, 90% MeOH) to give a subfraction E-1. Subfraction E-1 was then subjected to Sephadex LH-20 CC, eluting by $CHCl_3$–MeOH, 1:1, followed by preparative HPLC (MeOH–H_2O, 65:35, 3.2 mL/min) to give compound **1** (1.5 mg). Compound **2** (5.8 mg) was isolated from Fr. F by a protocol of silica gel CC ($CHCl_3$–MeOH, 50:1), Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:1), and PTLC ($CHCl_3$–MeOH, 13:1) in sequence.

Euphordracunculin C (1). Colorless gum; $[\alpha]_D^{20}$ +66.67° (*c* 0.09, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 269 (3.95), 220 (3.91), 200 (3.92). IR (KBr, ν, cm^{-1}): 1741, 1624, 1424, 1371, 1282, 1239, 1126, 1026. ESI-MS (positive mode) *m/z:* 540 $[M + H]^+$, 562 $[M + Na]^+$, 578 $[M + K]^+$; HR-ESI-MS (positive mode) *m/z* 562.2415 $[M + Na]^+$ (calcd for $C_{30}H_{37}O_8NNa^+$, 562.2417). For ^1H and ^{13}C NMR, see Table 1.

The known compound was identified as 4-hydroxy-*N*-[3-(4-hydroxy-3-methoxyphenyl)-1-oxo-2-propen-1-yl]-3-methoxybenzamide (**2**) by comparison of experimental data with those previously reported [10].

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REFERENCES

1. Q. W. Shi, X. H. Su, and H. Kiyota, *Chem. Rev*., **108**, 4295 (2008).
2. J. S. Ma and Y. Q. Cheng, *Editorial Committee of Flora Reipublicae Popularis Sinicae. Flora of China*, **44**, Science Press, Beijing, China, 1997, p. 118.

3. A. M. Zaghloul, *Mansoura J. Pharm. Sci.*, **9**, 204 (1993).
4. A. Singh and S. N. Srivastava, *Ind. J. Chem.*, **4**, 420 (1966).
5. L. Wang, Z. Zang, Y. F. Wang, S. X. Huang, P. Cao, and Y. Zhao, *Chin. Chem. Lett.,* **26**, 121 (2015).
6. W. Jiao, W. W. Dong, Z. F. Li, M. C. Deng, and R. H. Lu, *Bioorg. Med. Chem*., **17**, 4786 (2009).
7. A. M. Ayatollahi, M. Ghanadian, S. Afshaypuor, M. I. Choudhary, F. Koubarfard, and M. Rahmati, *Fitoterapia*, **81**, 891 (2010).
8. G. Appendino, E. Belloro, G. C. Tron, V. Jakupovic, and M. Ballero, *J. Nat. Prod*., **62**, 1399 (1999).
9. G. Appendino, G. Cravotto, T. Jarevang, and O. Sterner, *Eur. J. Org. Chem*., **2000**, 2933 (2000).
10. E. Byun, G. S. Jeong, R. B. An, T. S. Min, and Y. C. Kim, *Arch. Pharm. Res*., **33**, 67 (2010).