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Original article

Two new myrinsol diterpenoids from *Euphorbia dracunculoides* Lam.

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ABSTRACT

Two new myrinsol diterpenoids, euphordracunculins A (**1**) and B (**2**), together with three known analogues, euphorprolitherin B (**3**), proliferins A (**4**) and B (**5**), were isolated from the petroleum ether extract of the aerial parts of *Euphorbia dracunculoides* Lam. Their structures were elucidated by means of extensive spectroscopic analysis (NMR and ESI-MS) and comparison with data reported in the literature.

1. Introduction

The genus *Euphorbia* is the largest in the spurge family, comprising of more than 2000 species, many of which have been used in folk as traditional Chinese medicine for the treatment of skin diseases, edemas, *etc.* [1]. Previous investigations on this genus revealed that the major bioactive constituents are diterpenoids [2–5]. *Euphorbia dracunculoides* Lam., as an annual or short-lived perennial herb, is distributed in riverbanks, valleys and roadsides of sandy areas in North Africa, South Europe, and Southwest Asia [6], and has been used as a folk medicine in India for its purgative and diuretic effects [7]. The earlier phytochemical investigations on *E. dracunculoides* are limited to the presence of flavonoids [7–9], triterpenoids [9], and coumarins [10]. To the best of our knowledge, there are no reports about diterpenoids from *E. dracunculoides* over the last two decades. In our efforts to search for structurally interesting and potential bioactive diterpenoids from genus *Euphorbia*, two new myrinsol diterpenoids euphordracunculins A (**1**) and B (**2**), together with three known analogues, euphorprolitherin B (**3**) [11], proliferins A (**4**) and B (**5**) [12], were isolated from the aerial parts of *E. dracunculoides*. In this paper, the isolation and structural elucidation of two new compounds are presented (Fig. 1).

2. Experimental

2.1. Plant material

The aerial parts of *E. dracunculoides* Lam. were collected in September 2012 from Xishuang Banna prefecture, Yunnan Province, People's Republic of China, and identified by Prof. Yao-Wen Yang, Yunnan University of Traditional Chinese Medicine. A voucher specimen (YTCM 20120915) was deposited at Yunnan University of Traditional Chinese Medicine.

2.2. Extraction and isolation

The air-dried and powdered aerial parts of *E. dracunculoides* Lam. (4.0 kg) were extracted with 70% aqueous acetone (8 L × 2 d × 3) at room temperature. The extracts were concentrated by a rotary evaporator under reduced pressure to remove organic solvent. The aqueous residue was then partitioned with petroleum ether (4 × 1 L), EtOAc (4 × 1 L), and *n*-BuOH (4 × 1 L) sequentially. The petroleum ether extract (48.0 g) was subjected to column chromatography (CC) on silica gel (200–300 mesh) using a gradient system of increasing polarity with petroleum ether–EtOAc (50:1, 20:1, 10:1, 5:1, 2:1, 1:1, 0:1) to afford six fractions (A–F) based on TLC analysis.

Fraction D (7.2 g) was decolorized on a MCI gel (CHP 20P) CC eluted by MeOH, and then divided into three subfractions (Fr. D-1–Fr. D-3) by silica gel (200–300 mesh) CC eluting with petroleum ether/Me_2CO (15:1, 6:1, 1:1, respectively). Fr. D-1 was separated

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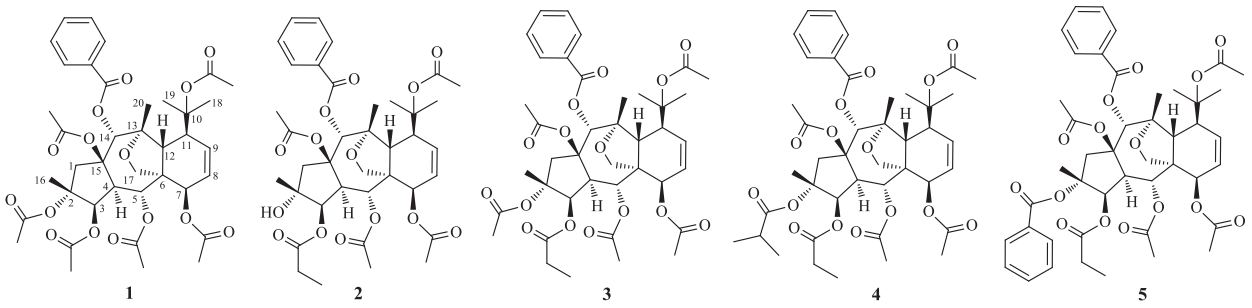

Fig. 1. Structures of compounds **1**–**5**.

by Sephadex LH-20 column (MeOH/$CHCl_3$, 1:1), followed by semipreparative HPLC (HITACHI HPLC system; YMC–Triart C_{18} column, 250 mm × 10 mm; DAD detector, MeOH/H_2O 75:25, 232 nm, 3.5 mL/min) to give compounds **3** (18.6 mg, t_R = 25.8 min), **4** (9.0 mg, t_R = 37.1 min), and **5** (3.2 mg, t_R = 40.1 min). Fr. D-2 was chromatographed on Sephadex LH-20 column (MeOH), followed by preparative HPLC (CXTH LC–3000 HPLC system, Kromasil C_{18} column, 250 mm × 20 mm; UV detector, MeOH–H_2O 76:24, 232 nm, 12.0 mL/min) to yield compound **2** (14.5 mg, t_R = 13.8 min). Fr. D-3 was subjected to Sephadex LH-20 CC eluted by MeOH, followed by semipreparative HPLC (HITACHI HPLC system; YMC–Triart C_{18} column, 250 mm × 10 mm; DAD detector, MeOH–H_2O 77:23, 232 nm, 3.5 mL/min) to afford compound **1** (8.6 mg, t_R = 15.9 min).

Table 1
NMR data for compounds **1** and **2** (TMS as the internal standard, δ in ppm, J in Hz).[a,b]

No.	**1**		**2**	
	δ_H J (Hz)	δ_C	δ_H J (Hz)	δ_C
1α	3.29 (d, 1H, J = 17.4, 1.1)	47.0 (t)	2.45 (dd, 1H, J = 16.4, 1.2)	51.2 (t)
1β	2.37 (d, 1H, J = 17.4)		2.27 (d, 1H, J = 16.4)	
2		87.1 (s)		78.4 (s)
3	5.36 (br d, 1H, J = 4.1)	78.5 (d)	5.12 (br d, 1H, J = 3.6)	80.4 (d)
4	3.72 (dd, 1H, J = 11.1, 4.1)	47.5 (d)	4.04 (dd, 1H, J = 11.1, 3.6)	47.1 (d)
5	5.95 (dd, 1H, J = 11.1, 1.5)	68.7 (d)	5.94 (dd, 1H, J = 11.1, 1.0)	68.7 (d)
6		53.7 (s)		53.7 (s)
7	4.86 (d, 1H, J = 6.6)	63.0 (d)	4.86 (d, 1H, J = 6.6)	63.1 (d)
8	6.18 (ddd, 1H, J = 9.9, 6.6, 1.5)	125.9 (d)	6.17 (ddd, 1H, J = 9.8, 6.6, 1.2)	125.9 (d)
9	5.91 (dd, 1H, J = 9.9, 5.6)	130.1 (d)	5.90 (dd, 1H, J = 9.8, 5.6)	130.0 (d)
10		86.0 (s)		86.1 (s)
11	3.18 (m, 1H)	44.8 (d)	3.17 (m, 1H)	44.8 (d)
12	3.20 (br d, 1H, J = 3.3)	37.2 (d)	3.23 (br d,1H, J = 3.4)	37.2 (d)
13		89.9 (s)		89.7 (s)
14	5.81 (s, 1H)	73.3 (d)	5.87 (s, 1H)	72.9 (d)
15		90.2 (s)		90.1 (s)
16	1.33 (s, 3H)	18.9 (q)	1.10 (s, 3H)	23.0 (q)
17a	4.16 (d, 1H, J = 8.7)	70.0 (t)	4.17 (d, 1H, J = 8.8)	70.0 (t)
17b	3.53 (dd, 1H, J = 8.7, 1.6)		3.51 (dd, 1H, J = 8.8, 1.2)	
18	1.64 (s, 3H)	25.3 (q)	1.64 (s, 3H)	25.3 (q)
19	1.55 (s, 3H)	21.3 (q)	1.54 (s, 3H)	21.3 (q)
20	1.22 (s, 3H)	24.5 (q)	1.24 (s, 3H)	24.7 (q)
2-OAc		169.5 (s)		
	1.71 (s, 3H)	22.5 (q)		
3-OAc		170.6 (s)		
	2.06 (s, 3H)	21.4 (q)		
3-OPr				174.0 (s)
1′			2.37 (1H, overlap)	28.2 (t)
			2.32 (1H, overlap)	
2′			1.15 (t, 3H, J = 7.5)	9.0 (q)
5-OAc		169.6 (s)		169.4 (s)
	2.00 (s, 3H)	21.1 (q)	1.98 (s, 3H,)	21.1 (q)
7-OAc		170.7 (s)		170.6 (s)
	1.98 (s, 3H)	21.2 (q)	1.97 (s, 3H)	21.0 (q)
10-OAc		170.9 (s)		170.9 (s)
	2.10 (s, 3H)	22.7 (q)	2.12 (s, 3H)	22.7 (q)
14-OBz		165.9 (s)		165.6 (s)
1″		130.0 (s)		129.4 (s)
2″, 6″	8.08 (d, 2H, J = 7.8)	130.2 (d)	8.20 (d, 2H, J = 7.3)	130.4 (d)
3″, 5″	7.45 (t, 2H, J = 7.8)	128.6 (d)	7.46 (t, 2H, J = 7.3)	128.8 (d)
4″	7.58 (t, 1H, J = 7.8)	133.5 (d)	7.59 (t, 1H, J = 7.3)	133.7 (d)
15-OAc		168.7 (s)		169.0 (s)
	2.15 (s, 3H)	22.5 (q)	2.13 (s, 3H)	22.5 (q)

[a] ^1H NMR and ^{13}C NMR data were recorded in $CDCl_3$ at 600 MHz and 150 MHz, respectively.
[b] The assignments were based on HSQC, ^1H–^1H COSY, HMBC, and ROESY experiments.

3. Results and discussion

Compound **1**, $[\alpha]_D^{26.5}$ −134.0 (*c* 0.21, MeOH), UV (MeOH) λ_{max} (log ε): 271 (2.98), 230 (4.08) and 201 (4.07) nm, obtained as colorless needles from MeOH, mp 197–200 °C. Its molecular formula was determined to be $C_{39}H_{48}O_{15}$ based on the HR-ESI-MS data (*m/z* 779.2887 $[M+Na]^+$, calcd. 779.2891), corresponding to 16 degrees of unsaturation. Its IR spectrum showed absorption bands for carbonyl groups at 1742 cm^{-1}. The ^1H NMR spectrum (Table 1) showed 10 3H-singlets at δ_H 2.15, 2.10, 2.06, 2.00, 1.98, 1.71, 1.64, 1.55, 1.33 and 1.22, of which six may be assigned for acetate groups and four be assigned for tertiary methyl groups. A mono-substituted benzene ring [δ_H 8.08 (d, 2H, *J* = 7.8 Hz), 7.58 (t, 1H, *J* = 7.8 Hz), 7.45 (t, 2H, *J* = 7.8 Hz)] was also evident in the ^1H NMR spectrum. Additionally, the signals of two vicinal olefinic protons [δ_H 6.18 (ddd, 1H, *J* = 9.9, 6.6, 1.5 Hz), 5.91 (dd, 1H, *J* = 9.9, 5.6 Hz)] and an oxygenated methylene group [δ_H 4.16 (d, 1H, *J* = 8.7 Hz), 3.53 (dd, 1H, *J* = 8.7, 1.6 Hz)] were also observed. Seven carbonyl signals at δ_C 170.9, 170.7, 170.6, 169.6, 169.5, 168.7 and 165.9, were obvious in the ^{13}C NMR spectrum of **1**. Accordingly, **1** was presumably a highly oxidized tetracyclic diterpenoid substituted by six acetoxy and one benzoyloxy groups. Four oxymethine protons geminal to ester functions [δ_H 5.95 (dd, 1H, *J* = 11.1, 1.5 Hz), 5.81 (1H, s), 5.36 (br d, 1H, *J* = 4.1 Hz), 4.86 (d, 1H, *J* = 6.6 Hz)] suggested that the other three ester groups were located at quaternary carbons. Comparison the above NMR data with those of euphorprolitherin B (**3**), a myrsinol diterpene [11] which was also isolated in our present study, indicated that they are quite structurally similar. The possible difference was that a propionyloxy group at C-3 in euphorprolitherin B is replaced by an acetoxy group in **1**, which was supported by the disappearance of the NMR signals of propionyloxyl and presence of a typical acetoxyl signals (δ_C 170.7, s, 21.4, q; δ_H 2.06, s) in **1**. The hypothesis was further verified by the HMBC correlations (Fig. 2) from a methyl signal at δ_H 2.06 (s, 3H) and an oxymethine proton signal at δ_H 5.36 (br d, 1H, *J* = 4.1 Hz, H-3) to an ester carbonyl signal at δ_C 170.7, respectively. The accurate assignments of all protons and carbons were preformed through the correlations in 2D-NMR spectra (^1H–^1H COSY, HSQC and HMBC) of **1** (Fig. 2), from which the positions of the ester groups were also clarified. The correlations of the protons at δ_H 5.95 (H-5), 4.86 (H-7), and 5.81 (H-14) with the carbonyl carbons at δ_C 169.6, 170.7, and 165.9 in the HMBC spectrum demonstrated the presences of two acetoxy and one benzoyloxy groups at C-5, C-7, and C-14, respectively. In addition, three slightly weak correlations from methyl signals in acetoxy groups at δ_H 1.71 (s, 3H, 2-OAc), 2.10 (s, 3H, 10-OAc), 2.15 (s, 3H, 15-OAc) to three quaternary carbons at δ_C 87.1 (s, C-2), 86.0 (s, C-10), 90.2 (s, C-15) (Fig. S5 in Supporting information), respectively, indicated that the three acetoxy groups were located at C-2, C-10 and C-15, respectively.

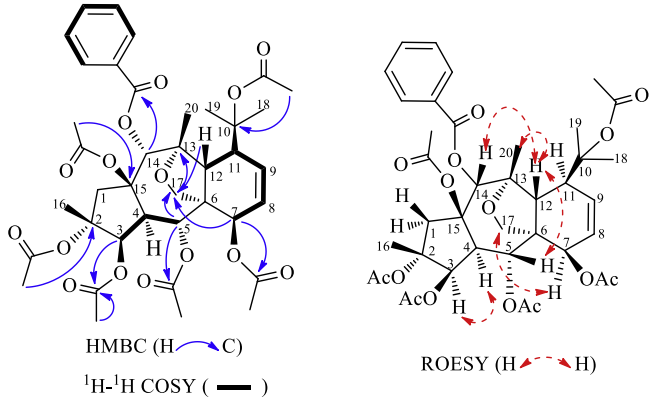

Fig. 2. Key COSY, HMBC, and ROESY correlations of **1**.

The relative configurations of **1** were decided by the ROESY experiment (Fig. 2) as well as biosynthetic consideration. For the reported natural myrsinol diterpenes, the three rings (5/7/6) forming the myrsinol diterpenoids' skeleton are *trans*-fused, H-4 and H_2-17 are α-oriented, and Me-16, H-12, the side chain at C-11, and the C-15 acyloxy group are β-oriented [13]. In addition, the ROESY correlations: H-4α with H-3 and H-7 with H-17a, b supported the α-orientations for H-3 and H-7, respectively, and the ROESY correlations between H-12β with H-5, H-14 and Me-20 were in agreement with the β-orientations of H-5, H-14 and Me-20 (Fig. S8 in Supporting information). Consequently, compound **1** was elucidated as 14-desoxo-2α,3β,5α,7β,10,15β-*O*-hexacetyl-14α-*O*-benzoyl-10,18-dihydromyrsinol and given the name euphordracunculin A.

Compound **2**, colorless crystals from MeOH, mp 199–203 °C, $[\alpha]_D^{26.5}$ −119.0 (*c* 0.18, MeOH), UV (MeOH) λ_{max} (log ε): 272 (3.06), 231 (4.12) and 201 (4.10) nm, was found to possess the molecular formula $C_{38}H_{48}O_{14}$ from the HR-ESI-MS data (*m/z* 751.2946 $[M+Na]^+$, calcd. 751.2942), indicating 15 degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **2** were similar to those of the known compound **3**, except for the absence of an acetoxyl group at C-2. A hydroxyl group at the C-2 position was evident for **2** on the basis of the observation of an upfield shifted quaternary carbon signal (δ_C 78.4) in the ^{13}C NMR spectrum and HMBC correlations of H-1 (δ_H 2.45), H-3 (δ_H 5.12), and Me-16 (δ_H 1.10) with C-2 (δ_C 78.4) (Fig. S15 in Supporting information). Further 2D NMR experiments allowed a determination of **2** as 14-desoxo-5α,7β,10,15β-*O*-tetraacetyl-14α-*O*-benzoyl-2α-hydroxy-3β-*O*-propionyl-10,18-dihydromyrsinol and given the name euphordracunculin B.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2014.08.005.

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