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A New Premyrsinane-type Diterpenoid Polyester from *Euphorbia dracunculoides* Lam

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Abstract: Phytochemical investigation of the 70% aqueous acetone extract of *Euphorbia dracunculoides* Lam. afforded a new premyrsinane-type diterpenoid polyester, 3*β*-*O*-isobutyryl-5*α*-*O*-benzoyl-7*β*,13*β*-di-*O*-acetyl-17-*O*-nicotinoylpremyrsinol (**1**), and two known analogues, euphorbialoids C (**2**) and D (**3**). Their structures were elucidated by means of extensive spectroscopic analysis (NMR and ESI-MS) and comparison with data reported in the literature.

Keywords: *Euphorbia dracunculoides;* premyrsinane-type; diterpenoid polyester.

1. Introduction

Premyrsinane-type diterpenoids, which were thought of originating from lathyrane-type diterpenoids biosynthetically, contain a *gem*-dimethylcyclopropane ring [1]. Naturally occurring premyrsinane-type diterpenoids with a 5/6/7/3 fused polycyclic skeleton were found limited to the genus *Euphorbia* (Euphorbiaceae) [1,2]. *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 [3]. It has been used as a folk medicine in India as laxatives and diuretics for many years [4]. However, its phytochemical investigation on diterpenoids are lacking, only one diterpenoid, named euphorbol was reported in 1966 [5]. In our efforts to search for structurally variable and potential bioactive diterpenoids from *Euphorbia dracunculoides*, a new premyrsinane-type diterpenoid polyester (**1**), together with two known analogues, euphorbialoids C (**2**) and D (**3**) [6], were isolated from the aerial parts of *Euphorbia dracunculoides* Lam. In this paper, we present the isolation and structural elucidation of the new compound (**1**).

2. Materials and Methods

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2.1. General procedures

Optical rotations were recorded using a JASCO P-1020 Polarimeter. UV spectra were recorded on Shimadzu UV-2401PC UV-VIS spectrophotometer. IR spectra were measured on a Bruker Tensor 27 FTIR Spectrometer (KBr). ^{1}H NMR, ^{13}C NMR and 2D NMR spectra were recorded in $CDCl_3$ using a Bruker AVANCE III-600 spectrometer or a Bruker DRX-400 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. Column chromatography (CC) was performed using silica gel (200–300 mesh, Qingdao Marine Chemical Inc.), Sephadex LH-20 (25–100 μm, Pharmacia Biotech Ltd.) and MCI gel CHP 20P (75–150 μm, Mitsubishi Corp.). Thin-layer chromatography (TLC) was performed using precoated silica gel GF_{254} plates (Qingdao Marine Chemical Inc.) with various solvent systems. Semipreparative HPLC was conducted on a HITACHI Chromaster system (Hitachi Ltd.) equipped with an YMC-Triart C_{18} column (250 mm × 10 mm i.d., 5 μm, YMC Corp.), using a flow rate of 3.5 mL/min at a column temperature of 25 °C, and the detection was performed with a DAD detector.

2.2. Plant Material

The material of plant (*Euphorbia dracunculoides* Lam.) was collected in the Xishuang Banna prefecture, Yunnan Province, People's Republic of China, in October 2012. A voucher specimen (YTCM 20121023) was deposited at the Yunnan Traditional Chinese Medical College, and was identified by Prof. Yao-wen Yang.

2.3. Extraction and Isolation

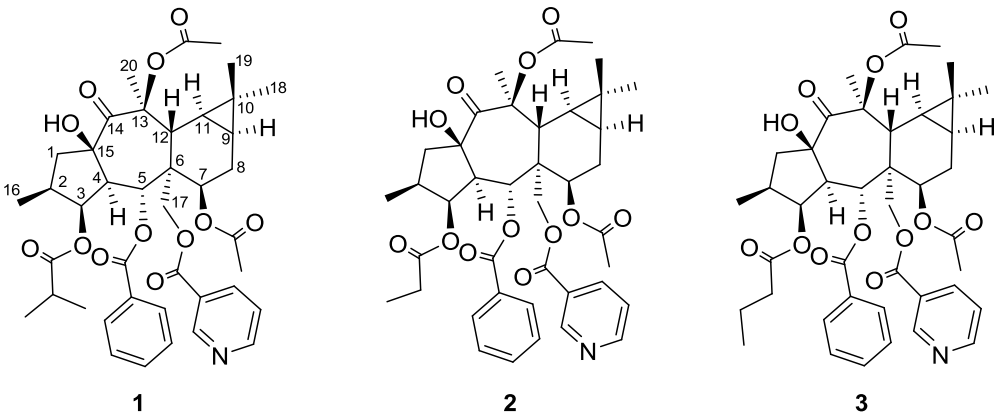
The air-dried and powdered aerial parts of *E. dracunculoides* Lam. (2.0 kg) were extracted with 70% aqueous acetone (8 L × 2 d × 3) at room temperature. The extracts were concentrated by a rotary evaporator under reduced pressure to remove organic solvent. The aqueous residue was then partitioned with EtOAc (4 × 1 L). The EtOAc layer (38.0 g) was then subjected to column chromatography (CC) on silica gel (200−300 mesh) using a gradient system of increasing polarity with petroleum ether−acetone (50:1, 20:1, 10:1, 5:1, 2:1, 1:1 and 0:1) to afford seven fractions (A−G) based on TLC analysis.

Fraction E (1.4 g) was decolorized on a MCI gel (CHP 20P) CC eluted by 90% CH_3OH–H_2O, then 100% MeOH. The 90% MeOH fraction (746.0 mg) was repeatedly chromatographed on Sephadex LH-20 column (MeOH−$CHCl_3$, 1:1) to give three subfractions, the third of which was further purified by semipreparative HPLC (MeCN–H_2O, 64:36), and yielded compounds **1** (9.0 mg, t_R = 29.8 min), **2** (33.5 mg, t_R = 20.2 min) and **3** (46.4 mg, t_R = 27.6 min).

3. Results and Discussion

Compound **1**, $[\alpha]_D^{26.5}$ –29.5 (*c* 0.34, MeOH), UV (MeOH) λ_{max} (log ε): 264 (3.50), 224 (4.19) and 201 (4.20) nm, obtained as white powder from MeOH. Its molecular formula was determined as $C_{41}H_{49}NO_{12}$ by HR-ESI-MS (*m/z* 770.3148 $[M+Na]^+$, calcd. 770.3152), corresponding to 18 degrees of unsaturation. The IR spectrum displayed the absorptions for the hydroxyl group at 3440 cm^{-1} and ester carbonyl groups at 1726 cm^{-1}. The ^{1}H NMR spectrum (Table 1) showed five 3H-singlets at δ_H 2.15, 2.13, 1.77, 1.07 and 0.97, and three 3H-doublets at δ_H 1.13 (*J* = 7.1 Hz), 0.96 (*J* = 6.9 Hz), 0.86 (*J* = 6.6 Hz). Furthermore, a mono-substituted benzene ring [δ_H 7.69 (2H, d, *J* = 7.3 Hz), 7.16 (1H, t, *J* = 7.3 Hz), 7.03 (2H, t, *J* = 7.3 Hz)] and a mono-substituted pyridine ring [δ_H 8.83 (1H, s), 8.53 (1H, d, *J* = 4.0 Hz), 7.60 (1H, d, *J* = 7.9 Hz), 7.00 (1H, dd, *J* = 7.9, 4.0 Hz)] were also evident in the ^{1}H NMR spectrum. Taking the five ester carbonyl groups (δ_C 176.0, 170.8, 170.2, 165.1 and 164.9) into consideration, the presences of five acyl groups (two acetyl, one benzoyl, one isobutyryl, and one nicotinoyl groups) were unambiguous in **1**. Additionally, an intra-annular carboxyl (δ_C 204.5) and six oxygen-bearing carbon signals at δ_C 85.7, 84.3, 78.1, 70.6, 70.0 and 63.8 appeared in the ^{13}C NMR

spectrum of **1**. Since only five of the oxygen-bearing carbon were oxygenated by ester functions, no other acyloxy groups were observed in **1**, and an evident hydroxy signal (3440 cm^{-1}) was observed in the IR spectrum, it could be supposed that the substituent group at C-15 might be a hydroxyl group. This hypothesis was in accordance with the molecular formula and the unsaturation. Meanwhile, a upfield quaternary carbon (δ_C 18.6) in the ^{13}C NMR spectrum together with the signals at δ_H 1.07 s, 0.97 s, 0.78 m and 0.78 m, and δ_C 29.5 (q), 23.9 (d), 19.4 (d) and 15.0 (q), indicated the presence of a *gem*-dimethylcyclopropane subunit. All of the above evidences suggested the structure of **1** as a premyrsinane-type diterpenoid polyester [7].



1 **2** **3**

Figure 1. The chemical structures of compounds **1-3**

Comparison of the NMR data of **1** with those of euphorbialoid D (**3**), indicated that they are structurally similar. The possible difference was that a butyryloxy group at C-3 in **3** is replaced by an isobutyryloxy group in **1**, which was supported by the disappearance of the NMR signals for two butyryl-specified methylenes in **3** and presence of a typical isobutyryloxy signals (δ_C 176.0, s, 34.2, d, 19.1, q, 18.4, q; δ_H 2.48, 1H, m, 1.13, 3H, d, J = 7.1 Hz and 0.96, 3H, d, J = 6.9 Hz) in **1,** along with the coincidence of the same formula weight 747 (ESI-MS *m/z*: 748 $[M+H]^+$, 770 $[M+Na]^+$) for each. This hypothesis was further verified by the HMBC correlations (Fig. 2) from two methyl signals (δ_H 1.13 and 0.96) to a ester carbonyl signal at δ_C 176.0 and a methine signal at δ_C 34.2, respectively.

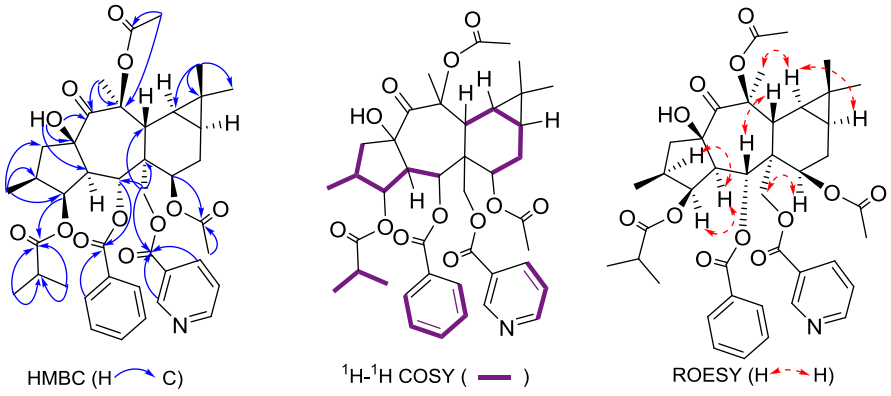


Figure 2. Key HMBC (left), COSY (middle) and ROESY (right) correlations of compound **1**

The accurate assignments of all protons and carbons were performed through the correlations in 2D-NMR spectra (^{1}H-^{1}H COSY, HSQC and HMBC) of **1** (Fig. 2), from which the positions of the acyloxy groups were also clarified. The correlations of the protons at δ_H 5.38 (H-3), 6.42 (H-5), 5.01 (H-7) and 5.04 (H-17a) with the carbonyl carbons at δ_C 176.0, 165.1, 170.2 and 164.9, respectively in the HMBC spectrum demonstrated the attachments of one isobutyryloxy, one benzoyloxy, one acetoxy and one nicotinoyloxy groups at C-3, C-5, C-7 and C-17, respectively. Moreover, a slightly weak correlation from a methyl signal in an acetoxy group at δ_H 2.13 (3H, s, 13-OAc) to a quaternary carbon at δ_C 85.7 (C-13) (Supporting Information, Fig. S5), indicated that an acetoxy group was located at C-

13. Additionally, HMBC correlations from a hydroxyl group δ_H 4.28 (1H, brs, OH-15) to the carbons at δ_C 50.7 (C-4), 84.3 (C-15) and 204.5 (C-14) (Fig. 2), respectively, indicated that C-15 was oxygenated by a hydroxyl group.

The relative configurations of **1** were elucidated as follows. For the reported natural premyrsinane-type diterpenoids, three *trans*-fused rings (5/6/7) construct the carbon skeleton, which restricts the *α*-orientations of H-4 and H_2-17 and *β*-orientations of H-12 and the substituent group at C-15 based on biosynthetic considerations [2, 8, 9]. ROESY correlations observed for H-2/H-4, H-3/H-4, H-5/H-12, H-7/H-17b, H-9/H-11 and H-11/Me-20 supported the conclusions that H-2, H-3, H-7, H-9, H-11 and Me-20 were all *α*-oriented, while H-5 was solely *β*-oriented. Consequently, compound **1** was determined as 3*β*-*O*-isobutyryl-5*α*-*O*-benzoyl-7*β*,13*β*-di-*O*-acetyl-17-*O*-nicotinoyl-premyrsinol.

Table 1. ^{1}H-NMR and ^{13}C-NMR data of compound **1** in $CDCl_3$[a, b]

No.	δ_H	δ_C	No.	δ_H	δ_C
1a	3.18 (1H, dd, *J* = 13.7, 7.8 Hz)	43.1 (t)	3-O*i*Bu		176.0 (s)
1b	1.67 (1H, d, *J* = 13.7 Hz)		1'	2.48 (1H, m)	34.2 (d)
2	1.88 (1H, m)	37.5 (d)	2'	0.96 (3H, d, *J* = 6.9 Hz)	18.4 (q)
3	5.38 (1H, t, *J* = 3.3 Hz)	78.1 (d)	3'	1.13 (3H, d, *J* = 7.1 Hz)	19.1 (q)
4	2.43 (1H, dd, *J* = 11.5, 3.8 Hz)	50.7 (d)	5-OBz		165.1 (s)
5	6.42 (1H, d, *J* = 11.5 Hz)	70.0 (d)	1'		129.4 (s)
6		48.2 (s)	2', 6'	7.69 (2H, d, *J* = 7.3 Hz)	129.6 (d)
7	5.01 (1H, d, *J* = 6.5 Hz)	70.6 (d)	3', 5'	7.03 (2H, t, *J* = 7.3 Hz)	128.1 (d)
8a	2.20 (1H, m)	22.4 (t)	4'	7.16 (1H, t, *J* = 7.3 Hz)	132.9 (d)
8b	1.93 (1H, m)		7-OAc		170.2 (s)
9	0.78 (1H, m)	19.4 (d)		2.15 (3H, s)	21.4 (q)
10		18.6 (s)	13-OAc		170.8 (s)
11	0.78 (1H, m)	23.9 (d)		2.13 (3H, s)	21.5 (q)
12	3.60 (1H, d, *J* = 6.3 Hz)	35.4 (d)	15-OH	4.28 (1H, brs)	
13		85.7 (s)	17-ONic		164.9 (s)
14		204.5 (s)	1'		125.2 (s)
15		84.3 (s)	2'	7.60 (1H, d, *J* = 7.9 Hz)	136.3 (d)
16	0.86 (3H, d, *J* = 6.6 Hz)	13.9 (q)	3'	7.00 (1H, dd, *J* = 7.9, 4.0 Hz)	123.2 (d)
17a	5.04 (1H, d, *J* = 11.6 Hz)	63.8 (t)	4'	8.53 (1H, d, *J* = 4.0 Hz)	153.1 (d)
17b	4.55 (1H, d, *J* = 11.6 Hz)		5'	8.83 (1H, s)	150.6 (d)
18	0.97 (3H, s)	15.0 (q)			
19	1.07 (3H, s)	29.5 (q)			
20	1.77 (3H, s)	25.1 (q)			

[a] ^{1}H NMR and ^{13}C NMR data were recorded in $CDCl_3$ at 600 MHz and 150 MHz, respectively.

[b] The assignments were based on DEPT, ^{1}H-^{1}H COSY, HSQC, HMBC and ROESY experiments.

According to the NMR and MS spectra as well as comparison with values from the literature [7], compounds **2** and **3** were identified as euphorbialoids C and D, respectively.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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