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DITERPENOIDS FROM *EUPHORBIA MICRACTINA*

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Key Word Index—*Euphorbia micractina*; Euphorbiaceae; diterpenoids; euphoractine C–E.

Abstract—Three new diterpenoids, euphoractine C–E, were isolated from *Euphorbia micractina*. Their structures were established on the basis of spectroscopic methods including 2D NMR techniques.

INTRODUCTION

In a preliminary publication, we reported on the isolation and structural elucidation of two new diterpene esters [euphoractine A (**1**) and B (**2**)] with a novel tetracyclic diterpene skeleton from the acetone extract of *Euphorbia micractina* [1]. The relative stereochemistry of euphoractine A was confirmed as **1** by X-ray analysis, however, the structure of euphoractine B was wrongly assigned, the reported spectral data of euphoractine B [1] revealed that **2** is the correct structure. ‡We now wish to report the structural determination of three further diterpene esters, euphoractine C (**3**), D (**4**) and E (**5**), from the same source.

RESULTS AND DISCUSSION

Euphoractine C (**3**) was obtained as prisms (EtOAc). It was assigned the molecular formula $C_{27}H_{36}O_6$ on the basis of elemental analysis and HR mass spectrometry. The IR spectrum of **3** exhibited the characteristic absorptions of hydroxyl groups (3489 cm^{-1}), carbonyls (1714 cm^{-1}) and an aromatic ring (1602 and 1499 cm^{-1}). In the EI mass spectrum the base peak at m/z 105 $[C_6H_5CO]^+$ suggested the presence of a benzoyl moiety, which was confirmed by the ^1H and ^{13}C NMR spectral data (Tables 1 and 2). In addition, the ^{13}C NMR spectrum indicated that the remaining part consisted of 20 carbons corresponding to Me(×5), CH_2(×3), CH(×7) (three CH–O, δ74.9, 73.6 and 63.7) and five quaternary carbons (an oxygenated, δ93.8; and a carbonyl, δ205.2). Thus, **3** is a benzoyl diterpene ester [2, 3].

The ^1H NMR spectrum showed four methyl singlets at δ1.16, 0.91, 0.78 and 0.37, and one methyl doublet at δ1.01 (J = 7.5 Hz), two doublets at δ5.06 (J = 11.5 Hz) and 3.36 (J = 8.7 Hz), and one double doublet at δ4.57 (J = 5.5 and 5.5 Hz) which were due to three oxymethines. A comparison of the ^1H and ^{13}C NMR spectral data with those of euphoractine A (**1**) suggested a benzoyl moiety at C-15 instead of the C-15 cinnamoyl moiety of **1**. The close spatial proximity of the benzoate to Me-19 and H_3-19 caused an unusual high field [3] shift of H-19 (δ0.37) in the ^1H NMR spectrum of **3**.

The ^1H and ^{13}C NMR spectral chemical shifts of **3**–**5** were assigned by 2D NMR techniques (^1H–^1H COSY, ^{13}C–^1H COSY and COLOC).

Euphoractine D (**4**), prisms (EtOAc), showed almost identical IR and EI mass spectral data to those of **3**. The ^1H NMR spectral data of **4** differ from those of **3** mainly in the chemical shifts and couplings of H-1–H-3 and H-16 (Table 1). In the ^{13}C NMR spectrum, the chemical shifts of C-1 to C-4 and C-16 of **4** were clearly different from those of **3** (Table 2). These facts suggested that **4** is a epimer of **3** at C-2 [4], which was confirmed by a NOE experiment. Irradiation of H-3 gave enhancements of H-4 (6%) and H-16 (9%), and irradiation of H-2 gave a 11% enhancement of H-1β.

Euphoractine E (**5**), gum, showed a molecular formula of $C_{29}H_{36}O_5$ from HR mass spectrometry. Its IR spectrum revealed the characteristic absorptions of hydroxyl (3447 cm^{-1}) and carbonyl (1719 and 1694 cm^{-1}) groups, and an aromatic ring (1579 and 1496 cm^{-1}). The ^1H and ^{13}C NMR spectra suggested the presence of a cinnamoyl moiety (Tables 1 and 2). In addition, the ^{13}C NMR and DEPT spectra indicated that the remaining moiety consisted of 20 carbons: Me(×5), CH_2(×3), CH(×6) (three CH-O) and six quaternary carbons. A quaternary carbon at δ21.2 indicated a gem-dimethyl three-membered ring [5, 6]. Two quaternary sp^2 carbon signals at δ157.5 and 137.7, and the conjugated carbonyl signal at δ199.1 indicated a $\Delta^{4,15}$ double bond. In the ^1H NMR spectrum the doublet at δ5.29 indicated that the cinnamoyl moiety was located at C-12α of the diterpene moiety, and the doublet at δ4.80 (1H, J = 6.4 Hz) and the singlet at δ5.20 (1H) indicated the presence of a hydroxyl group at C-3 and C-5. The relative stereochemistry of **5** was deter-

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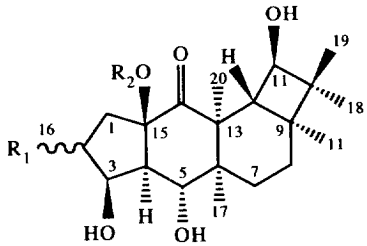
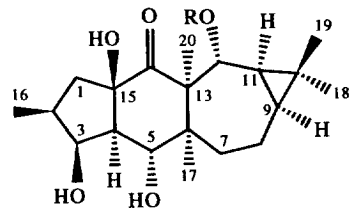
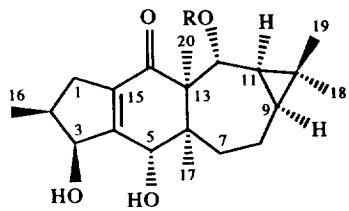


	R^1	R^2
1	βMe	cinnamoyl
3	βMe	benzoyl
4	αMe	benzoyl



2 R=cinnamoyl



5 R=cinnamoyl

Table 1. ^{1}H NMR spectral data of euphoractine C–E (**3–5**, 400.13 Hz, $CDCl_3$, TMS)

H	3	4	5
1α	2.63 *dd* (15.2, 11.3)	2.75 *dd* (14.5, 7.5)	2.64 *m*
1β	2.10 *dd* (15.2, 4.5)	1.65 *dd* (14.5, 10.7)	2.32 *m*
2	2.48 *m*	2.40 *m*	2.35 *m*
3	4.57 *dd* (5.5, 5.5)	4.20 *dd* (6.6, 2.4)	4.80 *d* (6.4)
4	2.05 *dd* (11.5, 5.5)	2.15 *dd* (11.5, 6.6)	—
5	5.06 *d* (11.5)	5.02 *d* (11.5)	5.20 *brs*
7	2.20 *m*	2.20 *m*	1.93 *m*
7′	1.18 *m*	1.18 *m*	1.70 *m*
8	1.43 *m*	1.43 *m*	1.56 *m*
8′	1.43 *m*	1.43 *m*	1.51 *m*
9	1.15 *m*	1.15 *m*	0.83 *m*
11	3.36 *d* (8.7)	3.36 *d* (8.7)	0.76 *m*
12	2.50 *dd* (12.0, 8.7)	2.50 *dd* (11.9, 8.7)	5.29 *d* (9.1)
16	1.01 *d* (7.5)	1.03 *d* (6.7)	1.08 *d* (7.1)
17	0.78 *s*	0.78 *s*	0.83 *s*
18	0.91 *s*	0.93 *s*	1.14 *s*
19	0.37 *s*	0.36 *s*	1.04 *s*
20	1.16 *s*	1.16 *s*	1.40 *s*
acid moiety			
2″/6″	8.10 *dd* (7.2, 1.1)	8.12 *dd* (7.2, 1.0)	7.50 *m*
3″/5″	7.46 *ddd* (7.2, 7.2, 0.4)	7.45 *ddd* (7.2, 7.2, 0.4)	7.35 *m*
4″	7.58 *m*	7.58 *m*	7.35 *m*
7″	—	—	7.59 *d* (16.0)
8″	—	—	6.32 *d* (16.0)

Coupling constants (J in Hz) are given in parentheses.

Table 2. ^{13}C NMR spectral data of euphoractine C–E (**3–5**, 100.62 Hz, $CDCl_3$, TMS)

C	3	4	5	Multiplicity
1	38.8	39.6	35.4	*t*
2	35.3	41.8	35.8	*d*
3	74.9	79.6	78.3	*d*
4	55.6 *d*	53.9 *d*	157.5 *s*	
5	63.7	63.8	65.0	*d*
6	47.4	47.6	47.6	*s*
7	32.7	32.6	32.7	*t*
8	22.0	22.0	19.9	*t*
9	39.3	39.3	25.0	*d*
10	41.5	41.6	21.2	*s*
11	73.6	73.6	29.5	*d*
12	48.8	48.7	71.0	*d*
13	56.7	56.6	60.7	*s*
14	205.2	205.7	199.1	*s*
15	93.8	93.4	137.7	*s*
16	16.1	19.5	15.9	*q*
17	18.0	18.1	18.1	*q*
18	27.9	27.9	28.4	*q*
19	14.2	14.2	13.6	*q*
20	13.7	13.6	11.9	*q*
Acid moiety				
1″	130.3	130.2	134.2	*s*
2″	129.9	130.1	128.8	*d*
3″	128.7	128.6	128.1	*d*
4″	134.6	133.5	130.5	*d*
5″	128.7	128.6	128.1	*d*
6″	129.9	130.1	128.8	*d*
7″	166.5 *s*	166.6 *s*	145.8 *d*	
8″	—	—	118.9	*d*
9″	—	—	166.0	*s*

mined by a difference NOE experiment. Irradiation of H-3 gave a 19% enhancement of H-2, on irradiation of H-5 a 7% enhancement of H-7′ was observed, and irradiation of H-12 gave a 15% enhancement of H_3-19. Consequently, the structure of euphoractine E was assigned as **5**.

EXPERIMENTAL

General. Mps: uncorr.; IR: KBr; ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.62 MHz). TMS as int. standard and $CDCl_3$ as solvent; HRMS and EIMS: 70 eV. All solvents were re-distilled prior to use.

Plant material. Euphorbia micractina was collected in Maqu Gansu Province of China in September 1990 and identified by Associate Prof. Zhi-Li Zhao. A voucher specimen (no. 9043) was deposited at the Herbarium in the Department of Pharmacy, Lanzhou Medical College.

Extraction and isolation. Air-dried powdered whole plants of *E. micractina* (10 kg) were extracted with Me_2CO at room temp., and the extract concd to obtain a residue (345 g). The residue (250 g) was chromatographed on a silica gel column and eluted with a gradient of petrol (60–90°) and Me_2CO. The fr. petrol–Me_2CO (3:1) was repeatedly chromatographed on silica gel columns eluting with cyclohexane–EtOAc (3:1) to yield the following compounds in order of elution: euphoractine C (20 mg), D (17 mg), A (40 mg), E (14 mg) and B (24 mg).

Euphoractine C (**3**). Mp 214–216°, $[\alpha]_D^{24.5}$ +57.5° ($CHCl_3$; *c* 0.50). IR ν_{max} cm^{-1}: 3489, 3064, 1714, 1637, 1602, 1499, 1453, 1382, 1288, 1148, 1118, 1066, 1031, 994, 714; HRMS *m/z* 456.5846, $C_{27}H_{36}O_6$ requires: 456.5851. Analyt.: Found: C, 70.89; H, 7.98. $C_{27}H_{36}O_6$ requires: C, 70.96; H, 7.95. EIMS *m/z* (rel. int.): 456 $[M]^+$ (4), 438 $[M-H_2O]^+$ (10), 423 (7), 334 (19), 319 (37), 245 (10), 105 (100), 77 (50), 69 (27), 55 (34); ^{1}H and ^{13}C NMR: Tables 1 and 2.

Euphoractine D (**4**). Mp 212–214°, $[\alpha]_D^{24.5}$ +43.6 ($CHCl_3$; *c* 1.30). IR ν_{max} cm^{-1}: 3487, 3065, 2949, 2867, 1716, 1639, 1602, 1499, 1455, 1382, 1286, 1152, 1116, 1037, 995, 714; HRMS *m/z* 456.5839, $C_{27}H_{36}O_6$ requires: 456.5851. EIMS *m/z* (rel. int.): 456 $[M]^+$ (5), 439 (12), 401 (14), 383 (18), 334 (19), 319 (27), 105 (100), 77 (48), 69 (37) 55 (42); ^{1}H and ^{13}C NMR: Tables 1 and 2.

Euphoractine E (**5**). $[\alpha]_D^{24.5}$ +17.3° ($CHCl_3$; *c* 1.22). IR ν_{max} cm^{-1}: 3447, 3061, 2927, 2870, 1719, 1694, 1635, 1579, 1496, 1452, 1381, 1280, 1171, 1098, 978, 960, 768, 712; HRMS *m/z* 464.6056, $C_{29}H_{36}O_5$ requires: 464.6063. EIMS *m/z* (rel. int.): 464 $[M]^+$ (20), 446 $[M-H_2O]^+$ (14), 316 (8), 298 (64), 283 (33), 270 (18), 225 (18), 227 (12), 191 (42), 163 (61), 131 (100), 103 (100), 77 (48), 69 (33), 55 (38); ^{1}H and ^{13}C NMR: Tables 1 and 2.

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