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NOVEL TRICYCLIC DITERPENOIDS FROM *EUPHORBIA MICRACTINA*

JIAN-GONG SHI,[1] ZHONG-JIAN JIA,* and YU-XING CUI

Institute of Organic Chemistry, State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China

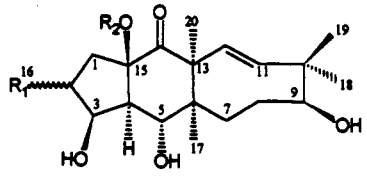
ABSTRACT.—From the Me_2CO extract of the whole plant of *Euphorbia micractina*, four new diterpenoids with a novel carbon skeleton, euphactins A–D [**1**–**4**], have been isolated. Their structures were elucidated by spectroscopic methods including 2D nmr techniques and X-ray crystallographic analysis.

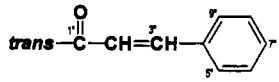
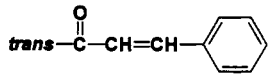
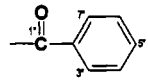
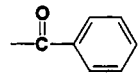
Euphorbia micractina Boiss. (Euphorbiaceae) is widely distributed at high altitudes (2700–5000 m) in western China, and is used in Chinese folk medicine for the treatment of tumors and warts (1). In previous papers on the constituents of this plant, we reported five diterpenoids, euphoractines A and B (2,3), and C, D, and E (4). We describe herein the isolation and structure elucidation of four closely related diterpenoids with a novel tricyclic carbon skeleton, euphactins A [**1**], B [**2**], C [**3**], and D [**4**], which were obtained from this same plant.

RESULTS AND DISCUSSION

The Me_2CO extract of the air-dried whole plant of *E. micractina* was subjected to cc on Si gel (200–300 mesh) to afford euphactins A [**1**], B [**2**], and a mixture of euphactins C [**3**] and D [**4**]. The mixture was further separated by hplc to give pure **3** and **4**.

Compound **1** was obtained as colorless prisms from Me_2CO. The ir spectrum of **1** showed absorption bands for hydroxyl groups (3455 cm^{-1}), carbonyl groups (1720 cm^{-1}), and an aromatic ring (1578, 1497, and 768 cm^{-1}). The eims of **1** exhibited a $[M]^+$



	R_1	R_2
1	β Me	
2	α Me	
3	β Me	
4	α Me	

[1]Present address: Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China.

at *m/z* 482, and hreims and elemental analysis gave a molecular formula of $C_{29}H_{38}O_6$. A base peak at *m/z* 131 $[C_6H_5CH=CHCO]^+$ and fragment peaks at *m/z* 334 $[M-C_6H_5CH=CH-COOH]^+$, 103 $[C_6H_5CH=CH]^+$, 77, 69, and 55 indicated the presence of a cinnamoyl moiety, which was confirmed by the ^{1}H- and ^{13}C-nmr spectral data of **1** (see Tables 1 and 2). In addition to the signals of the cinnamoyl moiety, the ^{13}C-nmr and DEPT spectra showed 20 carbons, including five methyls, three methylenes,

TABLE 1. ^{1}H-Nmr Data of Compounds **1–4** in $CDCl_3$.

Proton	Compound			
	1	**2**	**3**[b]	**4**[b]
H-1α	2.88 dd (15.3,11.3)	2.35 dd (14.5,7.9)	2.90 dd (15.4,11.2)	2.57 dd (14.7, 7.7)
H-1β	1.75 dd (15.3,4.1)	1.66 dd (14.5,10.2)	1.87 dd (15.4,4.2)	1.79 dd (14.7,10.0)
H-2	2.47 m	2.24 m	2.50 m	2.33 m
H-3	4.47 t (5.5)	4.05 dd (6.6,2.4)	4.53 t (5.3)	4.20 dd (6.9,2.1)
H-4	2.03 dd (11.5,5.5)	1.90 dd (11.6,6.6)	2.08 dd (11.5,5.3)	2.10 dd (11.5,6.9)
H-5	4.94 d (11.5)	4.80 d (11.6)	5.00 d (11.5)	4.98 d (11.5)
H-7	1.80 m	1.70 m	1.75 m	1.75 m
H-7′	0.95 br dd (16.2,6.3)	0.88 br dd (16.5,5.9)	0.96 br dd (16.2,6.1)	0.96 br dd (16.2,6.1)
H-8	1.82 m	1.73 m	1.77 m	1.77 m
H-8′	1.51 br t (13.3)	1.39 br t (13.3)	1.46 br t (13.4)	1.46 br t (13.4)
H-9	3.30 br d (9.7)	3.14 br d (9.6)	3.23 br d (9.8)	3.23 br d (9.8)
H-11	5.39 d (16.5)	5.30 d (16.6)	5.32 d (16.5)	5.32 d (16.5)
H-12	6.15 d (16.5)	6.16 d (16.6)	6.06 d (16.5)	6.07 d (16.5)
H-16	1.12 d (6.9)	1.14 d (7.2)	1.09 d (7.2)	1.17 d (7.0)
H-17	0.72 s	0.65 s	0.72 s	0.74 s
H-18	1.17 s	1.08 s	1.16 s	1.16 s
H-19	0.84 s	0.76 s	0.26 s	0.27 s
H-20	1.13 s	1.06 s	1.05 s	1.05 s
H-2″	6.50 d (15.9)	6.52 d (16.0)	—	—
H-3″	7.76 d (15.9)	7.82 d (16.0)	8.08 br d (7.0)	8.08 br d (7.0)
H-4″	—	—	7.47 br t (7.0)	7.49 br d (7.0)
H-5″	7.52 dd (8.1,1.7)	7.55 m	7.61 m	7.61 m
H-6″	7.39 dd (8.1,7.4)	7.46 m	7.47 br t (7.0)	7.49 br d (7.0)
H-7″	7.39 m	7.46 m	8.08 br d (7.0)	8.08 br d (7.0)
H-8″	7.39 dd (8.1,7.4)	7.46 m	—	—
H-9″	7.52 dd (8.1, 1.7)	7.55 m	—	—

[a]At 400.13 MHz, δ in ppm, *J* (in parentheses) in Hz.
[b]Assignments by comparison with **1** and **2** assisted by 2D COSY data.

seven methines (three CH-O at δ 84.0, 75.8, and 64.7; two olefinic carbons at δ 136.6 and 130.3), and five quaternary carbons (one oxygenated at δ 94.2; one carbonyl at δ 203.0); the ^{1}H-nmr spectrum showed four methyl singlets at δ 0.72, 0.84, 1.13, and 1.17, a methyl doublet at δ 1.12 (*J*=6.9 Hz), three oxymethine protons at δ 3.30 (1H, br d, *J*=9.7 Hz), 4.47 (1H, t, *J*=5.5 Hz) and 4.94 (1H, d, *J*=11.5 Hz), and two olefinic protons attributed to an isolated trans- double bond at δ 5.39 (1H, d, *J*=16.5 Hz) and 6.15 (1H, d, *J*=16.5 Hz). Taking into account the above data for a total of eight degrees of unsaturation, **1** was deduced as a tricyclic diterpenol cinnamate. Comparison of the ^{1}H- and ^{13}C-nmr spectra of **1** with those of euphoractine A, for which the structure was confirmed by X-ray crystallography (5), indicated that **1** has the same A and B rings as euphoractine A. The ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra of **1** revealed that the ring C contained the partial structures R-C^{7}H$_2$-C^{8}H$_2$-C^{9}HR-OH, CH$_3$-C^{10}R$_2$-CH$_3$ and *trans*-R-C^{11}H=C^{12}H-R (R=nonprotonated carbon atoms). A ^{1}H-^{13}C COLOC nmr experiment showed cross-peaks between C-6 (δ 43.0) and H$_2$-8 (δ 1.82 and 1.51) and H-12 (δ 6.15), between C-10 (δ 53.6) and H$_2$-8 and H-12, and between C-13 (δ 62.0) and H$_2$-

TABLE 2. ^{13}C-Nmr Data of Compounds **1–4** in $CDCl_3$.

Carbon	Compound			
	1[b]	**2**[b]	**3**[c]	**4**[c]
C-1	39.2	39.8	39.2	39.9
C-2	35.8	40.6	35.8	42.3
C-3	75.8	78.2	75.4	79.9
C-4	55.3	53.8	55.4	53.5
C-5	64.7	63.2	64.9	65.1
C-6	43.0	42.6	42.8	42.8
C-7	33.8	33.4	33.7	33.6
C-8	29.2	29.1	29.2	29.2
C-9	84.0	82.8	84.0	84.0
C-10	53.6	53.4	53.4	53.6
C-11	136.7	136.1	136.6	136.6
C-12	130.3	130.0	130.1	130.0
C-13	62.0	61.1	62.0	61.9
C-14	203.0	204.1	203.1	203.8
C-15	94.2	92.2	94.0	93.6
C-16	16.0	19.4	16.0	19.7
C-17	17.8	17.3	17.8	17.9
C-18	26.5	26.4	26.4	26.4
C-19	16.1	15.8	14.8	14.8
C-20	14.2	13.8	14.1	14.0
C-1″	165.3	165.2	165.1	165.2
C-2″	116.5	117.8	129.4	130.1
C-3″	147.8	145.8	128.7	128.8
C-4″	133.7	133.9	129.8	129.9
C-5″	128.4	127.7	133.8	133.7
C-6″	129.0	128.4	129.8	129.9
C-7″	131.1	129.8	128.7	128.8
C-8″	129.0	128.4	129.8	129.9
C-9″	128.4	127.7	133.8	133.7

[a]At 100.62 MHz, δ in ppm.
[b]Assignments from DEPT, ^{1}H-^{13}C COSY, and ^{1}H-^{13}C COLOC data.
[c]Assignments by comparison with **1** and **2**.

7 (δ 0.95, 1.80) and H-11 (δ 5.39), so that the tricyclic structure of **1** is constituted by a five-, six-, and eight-membered ring system, with the isolated trans- double bond occurring between C-11 and C-12, and a hydroxyl group at C-9. In the ^{1}H-^{1}H NOESY spectrum of **1**, a strong cross-peak between H-9 (δ 3.30) and Me-18 (δ 1.17) indicated a β- orientation of the hydroxy group at C-9. In addition, cross-peaks between H-11 (δ 5.39) and Me-18 (δ 1.17), and between H-12 (δ 6.15) and H-5 (δ 4.94) and Me-19 (δ 0.84), revealed the geometry of the double bond between C-11 and C-12 as *E*.

An X-ray crystallographic analysis of **1** confirmed the structure and relative stereochemistry assigned from the foregoing evidence. The crystal structure was solved by direct methods. A view of the solid-state conformation, with the atom numbering scheme indicated, is provided in Figure 1. Bond lengths are in accord with expected values (6).

Euphactin B [**2**], obtained as white prisms from Me_2CO, showed almost identical ir and eims spectral data to those of **1**. The most prominent differences in the ^{1}H-nmr spectrum of **2**, compared with that of **1**, were that a double doublet of **2** at δ 4.05 (1H, J=6.6 and 2.4 Hz) replaced the triplet of **1** at δ 4.47 (1H, J=5.5 Hz), and that the chemical shifts of the H-1α, H-1β, and H-2 signals of **2** were shifted upfield 0.53, 0.09,

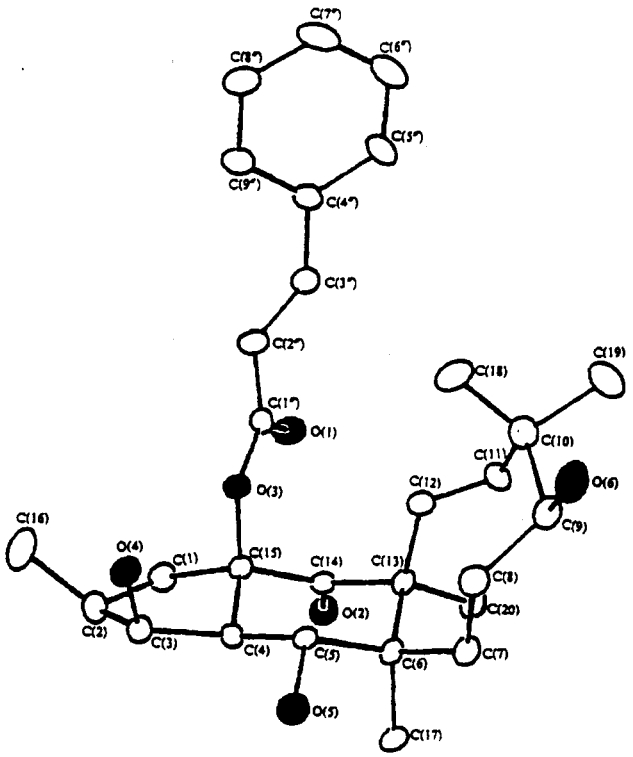


FIGURE 1. ORTEP diagram showing the atom numbering scheme and solid-state conformation of euphactin A [**1**]; Hydrogen atoms are omitted for clarity.

and 0.23 ppm, respectively. Furthermore, the coupling constants $J_{1\alpha,2}$ and $J_{1\beta,2}$ were changed from 11.3 and 4.1 Hz in **1** to 7.9 and 10.2 Hz in **2**. All the above data indicated that **2** is a Me-16α epimer of **1** (7). The complete assignments of the ^{1}H- and ^{13}C-nmr signals of **2** were based on ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY experiments. In the ^{1}H-^{1}H NOESY spectrum of **2**, cross-peaks between H-3 (δ 4.05), H-4 (δ 1.90), and Me-16 (δ 1.14) further confirmed the structural assignment.

Euphactin C [**3**] gave white prisms with Me_2CO. The ir spectrum of **3** displayed absorption bands for hydroxy groups (3447 cm^{-1}), carbonyl groups (1721 cm^{-1}) and an aromatic ring (1603 and 1479 cm^{-1}). The eims spectrum of **3** exhibited a molecular ion peak at *m/z* 456 [M$^+$], and hreims gave a molecular formula of $C_{27}H_{36}O_6$. A base peak at *m/z* 105 $[C_6H_5CO]^+$ and fragments at *m/z* 334 $[M-C_6H_5COOH]^+$, 77, 69, and 55 suggested the presence of a benzoyl moiety in the molecule. The ^{1}H-, ^{13}C-nmr, and DEPT spectra of **3** (Tables 1 and 2) resembled those of **1** except that the cinnamoyl moiety of **1** was replaced by the benzoyl moiety in the case of **3**, and that the Me-19 proton signal was shifted upfield from δ 0.84 of **1** to δ 0.26 of **3** from the shielding effect of the benzoyl moiety of **3** (8). Thus, the structure of euphactin C was assigned as **3**.

Euphactin D [**4**], white prisms (Me_2CO), showed almost identical ir and eims spectral data to those of **3**. Comparison of the ^{1}H-nmr spectrum of **4** with that of **3** indicated that the primary differences were that a double doublet in **4** at δ 4.20 (1H, J=6.9 and 2.1 Hz) replaced the triplet in **3** at δ 4.53 (1H, J=5.3 Hz), and that the chemical shifts of the H-1α, H-1β, and H-2 signals of **4** were shifted upfield 0.33, 0.08, and 0.17 ppm, respectively. Moreover, the coupling constants $J_{1\alpha,2}$ and $J_{1\beta,2}$ were changed

from 11.2 and 4.2 Hz in **3** to 7.7 and 10.0 Hz in **4**. From the above evidence, the structure in the **4** was assigned as a Me-16α epimer of **3**, which was supported by the differences in the ^{13}C-nmr spectral data between compounds **3** and **4** (see Table 2).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler melting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ with a Rudolph Research Autopol III automatic polarimeter. Ir spectra were recorded as KBr disks on a Nicolet 170SX Ft-ir instrument. Eims and hreims spectra were run on a VG ZAB-HS mass spectrometer (70 eV). ^{1}H-Nmr (400.13 MHz), ^{13}C-nmr (100.62 MHz), and 2D nmr spectra were determined on a Bruker AM-400 spectrometer in $CDCl_3$ with TMS as internal standard. All solvents used were analytical grade. Si gel 200–300 mesh was used for cc.

PLANT MATERIAL.—*E. micractina* was collected in Maqu, Gansu Province, People's Republic of China, in September 1990. It was identified by Mr. Zhili Zhao, Associate Professor of Plant Taxonomy, Department of Pharmacy, Lanzhou Medical College, Lanzhou, People's Republic of China. A voucher specimen (No. 9043) is deposited at the Herbarium of the Department of Pharmacy, Lanzhou Medical College.

EXTRACTION AND ISOLATION.—The air-dried powdered plant material (10 kg) was extracted with Me_2CO at room temperature, and the solvent was removed under reduced pressure at less than 40° to give a residue (345 g). A portion of the residue (250 g) was chromatographed on Si gel, eluting with petroleum ether (60–90°)/Me_2CO using a step gradient of increasing Me_2CO to afford seven fractions. The most polar fraction (petroleum ether-Me_2CO, 1:1) was repeatedly rechromatographed on Si gel using cyclohexane-Me_2CO (3:2) as eluent to yield pure euphactins A [**1**] (25 mg) and B [**2**] (18 mg), and a mixture of euphactins C and D. The mixture was further purified by hplc on a Whatman Partisil column eluting with cyclohexane-Me_2CO (3:2) to give euphactins C [**3**] (7 mg) and D [**4**] (5 mg).

Euphactin A [**1**].—Mp 252–254°; $[\alpha]^{24}D$ −120.0° (c=0.90, $CHCl_3$); ir (KBr) ν max 3455, 3060, 2928, 2850, 1720, 1634, 1578, 1497, 1452, 1363, 1337, 1281, 1204, 1174, 1133, 1062, 998, 768 cm^{-1}; eims *m/z* $[M]^+$ 482 (3), 464 (3), 356 (2), 351 (2), 334 (7), 316 (12), 301 (15), 288 (4), 273 (3), 259 (5), 191 (13), 175 (10), 163 (12), 148 (29), 147 (30), 131 (100), 103 (19), 77 (11), 69 (12), 55 (12); hreims *m/z* $[M]^+$ 482.6229 ($C_{29}H_{38}O_6$ requires 482.6234); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; *anal.* calcd for $C_{29}H_{38}O_6$, C 72.17, H 7.94, found C 72.11, H 7.89.

Euphactin B [**2**].—Mp 250–252°; $[\alpha]^{24}D$ 37.6° (c=0.10, $CHCl_3$); ir (KBr) ν max 3530, 3397, 3064, 2947, 2874, 1714, 1691, 1636, 1577, 1494, 1453, 1403, 1334, 1312, 1239, 1209, 1167, 1140, 1064, 1042, 1007, 978, 876, 773 cm^{-1}; eims *m/z* $[M]^+$ 482 (2), 464 (5), 356 (3), 351 (3), 334 (13), 316 (17), 301 (11), 288 (5), 259 (10), 191 (23), 175 (13), 163 (27), 148 (27), 147 (30), 131 (100), 103 (95), 77 (46), 69 (57), 55 (48); hreims *m/z* $[M]^+$ 482.6232 ($C_{29}H_{38}O_6$ requires 482.6234); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; *anal.* calcd for $C_{29}H_{38}O_6$, C 72.17, H 7.94, found C 72.14, H 7.96.

Euphactin C [**3**].—Mp 258–260°; $[\alpha]^{24}D$ −121.6° (c=0.54, $CHCl_3$); ir (KBr) ν max 3447, 3064, 2931, 2874, 1721, 1639, 1603, 1497, 1453, 1381, 1318, 1284, 1114, 1062, 998, 714 cm^{-1}; eims *m/z* $[M]^+$ 456 (4), $[M-H_2O]^+$ 438 (7), 423 (4), 334 (12), 319 (10), 316 (15), 259 (12), 191 (17), 154 (20), 123 (22), 105 (100), 77 (37), 69 (24), 55 (29); hreims *m/z* $[M]^+$ 456.5842 ($C_{27}H_{36}O_6$ requires 456.5851); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

Euphactin D [**4**].—Mp 259–261°; $[\alpha]^{24}D$ 32.8° (c=0.23, $CHCl_3$); ir (KBr) ν max 3441, 3066, 2933, 2873, 1722, 1640, 1601, 1499, 1453, 1383, 1319, 1284, 1114, 1061, 999, 716 cm^{-1}; eims *m/z* $[M]^+$ 456 (3), $[M-H_2O]^+$ 438 (5), 423 (2), 334 (12), 319 (15), 316 (15), 259 (14), 191 (15), 154 (20), 123 (25), 105 (100), 77 (48), 69 (37), 55 (42); hreims *m/z* $[M]^+$ 456.5849 ($C_{27}H_{36}O_6$ requires 456.5851); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

X-RAY CRYSTAL STRUCTURE ANALYSIS OF EUPHACTIN A[2] [**1**].—Crystal Data: $C_{29}H_{38}O_6$, mol wt=482.62, orthorhombic, space group $P2_12_12_1$, a=11.563 (1), b=12.597 (2), c=17.564 (2) Å, V=2558.1 (7) Å^3, Z=4, Dc=1.253 g/cm^{-3}, μ (CuKα radiation, λ=1.5418 Å)=6.6 cm^{-1}, crystal dimensions: 0.20×0.25×0.25 mm.

Preliminary unit-cell parameters and space group information were derived from oscillation and Weissenberg photographs. Intensity data (h>0, k>0, l>0; 1867 reflections) were recorded on an Enraf-

[2]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

Nonius CAD-4 diffractometer [CuKα radiation, graphite monochromator; ω-2θ scans, scan width (1.00+0.14 tan, θ)°, θ_{max}=75°]. The intensities of four reference reflections, monitored every 2 h during data collection, showed no significant variation (<1%). The data were corrected for the usual Lorentz and polarization effects. A total of 1237 reflections with $I>3\sigma$ (I) was retained for the analysis.

The crystal structure was solved by direct methods (MULTAN 11/82). Initial carbon and oxygen atom coordinates were obtained from an E map. A series of difference Fourier syntheses, evaluated following several rounds of full-matrix least-squares adjustment of positional and thermal parameters for these atoms (at first isotropic, then anisotropic), yielded hydrogen atom positions. With the inclusion of hydrogen atom positional and isotropic thermal parameters, and latterly an extinction correction (g), as variables in the subsequent least-squares iterations, the refinement converged (max. shift, ESD=0.03) at $R=\Sigma||F_o|-F_c||/\Sigma|F_o|=0.039$, $R_w=[\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2}=0.041$, $g=1.0(1)\times10^{-6}$, $GOF=[\Sigma w(|F_o|-|F_c|)^2/[(N_{observations}-N_{parameters})]^{1/2}=1.31$.

Crystallographic calculations were performed on PDP 11/44 and Micro VAX computers by use of the Enraf-Nonius Structure Determination Package (SDP). For structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were from the literature (*9*). In the least-squares iterations, $\Sigma\ w\Delta^2[w=1/\sigma^2(|F_o|), \Delta=(|F_o|-|F_c|)]$ was minimized.

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