Cytotoxic Macrocyclic Diterpenoids from *Euphorbia helioscopia*

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(Received July 22, 2008/Revised September 3, 2008/Accepted November 5, 2008)

A new cytotoxic macrocyclic diterpenoid, euphornin L (1), together with seven known analogues were isolated from the plant *Euphorbia helioscopia* L. The structure of 1 was elucidated by spectral data and X-ray crystallographic analysis. Euphorin L (1) and euphoscopin F (2) exhibited significant cytotoxicity against HL-60 cell lines with IC₅₀ values of 2.7 and 9.0 µM, respectively. The ¹³C-NMR data of euphoscopin F (2), epieuphoscopin B (3), euphoscopin B (5), and euphoscopin C (6) were also reported for the first time.

**Key words:** Diterpenoid, Euphorin L, X-ray, *Euphorbia helioscopia* L, Cytotoxicity

**INTRODUCTION**

The genus *Euphorbia* (Euphorbiaceae) mainly contains diterpenoid compounds that are responsible for the skin-irritating, tumor-promoting, and cytotoxic activities (Singla and Kamalal, 1990; Amir, 2006). *Euphorbia helioscopia* L. has been used to treat malaria, bacillary dysentery, osteomyelitis, and tumor in Chinese folk medicine (Hua et al., 1999; Gao, 1997). Up to now, only one obvious cytotoxic macrocyclic diterpenoid ester has been reported from this plant during the past three decades (Lu et al., 2008). Bioassay-guided phytochemical study on *E. helioscopia* revealed a new cytotoxic macrocyclic diterpenoid ester (1), together with seven known analogues (2-8). Compounds 1 and 2 exhibited significant cytotoxicity against HL-60 cell lines with IC₅₀ values of 2.7 and 9.0 µM, respectively. Since there were no reports in the literature, ¹³C-NMR data of compounds 2, 3, 5, and 6 were also reported in this paper.

**MATERIALS AND METHODS**

**General experimental procedures**

Melting points were determined on a Fisher-Johns apparatus and uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on Beckmen DU® 640 spectrophotometer. IR spectra were recorded using a Bruker model. 1D-NMR and 2D-NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard and chemical shifts were recorded as δ values. ESI-MS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semi-preparative HPLC was performed using ODS columns [YMC-pack ODS-A, 10 × 250 mm, 5 µm, 4.0 mL/min] in Waters 600 multisolvant delivery system equipped with a photodiode array detector (Waters, 996).

**Plant material**

The herbal drug “maoyancao” (*E. helioscopia*) was purchased as dried whole plants from a drug store in Henan Province of China. Botanical identification was carried out by Dr Yan Luo and the voucher specimen (Y.LUO613) was deposited in the College of Marine Life, Ocean University of China.

**Extraction and isolation**

The dried whole plant (4.5 kg) of *E. helioscopia* was powdered and extracted with 95% EtOH (3 × 10 L) to give a dark green residue (140.6 g) after removal of the solvent. The residue was distributed between light petroleum ether (3 L) and water (3 L) for 3 times, and the light petroleum ether layers afforded green oil (71.0 g) after removing the solvent. The green oil was subjected to column chromatography (CC, 10 × 50 cm) over macro-
porous resin (AB-8) and eluted with 50% EtOH (20 L),
80% EtOH (15 L) and 95% EtOH (15 L) successively.
The 80% EtOH elutions (15.0 g) were chromatographed
on silica gel (300-400 mesh, 250 g) and gradiently eluted
with petroleum ether-CHCl 3 and CHCl 3-MeOH to divide
into 11 fractions (Fraction 1-11). Fraction 8 (2.8 g) was
separated into 12 sub-fractions by CC on ODS (30 g)
eluting with MeOH-H 2O solutions (30-90%). Sub-fraction
5 gave compounds 3 (40.0 mg) and 5 (15.2 mg) after
being subjected to CC on Sephadex LH-20 eluted with
MeOH and HPLC eluted with 65% MeOH-H 2O. Com-
ponent 1 (25.0 mg) and 2 (12.5 mg) were obtained
from sub-fraction 6 through CC on Sephadex LH-20, silica gel
and HPLC in sequence. Fraction 9 (2.5 g) was separated
into 9 sub-fractions on ODS (28 g) and eluted with
MeOH-H 2O solutions (40-90%). Being chromatographed
over sephadex LH-20, silica gel and HPLC, sub-fraction
5 and 6 afforded compounds 4 (32.0 mg) and 8 (60.0
mg), respectively. Compound 6 (50.2 mg) was obtained
from sub-fraction 8 through CC on Sephadex LH-20 and
silica gel. Fraction 11 (1.5 g) was subjected to CC over
ODS, sephadex LH-20, silica gel, and HPLC to give
compound 7 (35.0 mg).

Euphorin L (1): colorless crystal (MeOH): m.p. 188-
190°C; [α] D 20 -108.2° (c 0.3, CHCl 3); UV (MeOH) λ max
(log ε) 225 (3.8), 273 (2.9) nm; IR (KBr) v max 2959,
1736, 1244, 750, 705 cm -1; 1H- and 13C-NMR (see Table
I); HRESI-MS m/z 649.3008 [M + Na] + (calcd. 649.2989
for C35H46O10Na).

X-ray crystal Structure of 1

The X-ray-diffraction data of compound 1 were collect-
ed on a Siemens SMART-CCD area-detector diffractometer
using the φ- and ω-scan technique (scan width 1.58-
25.01°; 2θ≤ 50°). A colorless prism of compound 1 (0.56
× 0.45 × 0.41 mm) obtained from MeOH was selected for
data collection, and the structure was solved by direct
methods and refined by block-matrix least-squares proce-
dure, using the SHELX-97 program system. H-positions
were found from difference Fourier maps and geometric
calculations. Crystal data: C35H46O10, M, 626.72 g mol -1;
absorption coefficient 0.083 mm -1; space group P(2)1;
triclinic, a = 10.9176(18), b = 18.069(3), c = 18.451(3)
Å, x = 90, β = 94.279 (2), γ = 90°; V = 3629.7(9) Å 3; Z = 4;
F(000) = 1344; D calc. = 1.147 g cm -3, T min/T max: 0.9548/
0.9666; data/restraints/ parameters 10586/1/812; goodness
of fit on F 2 : 1.002; R 1 and wR 2 [I≥2σ(I)]: 0.0560 and
0.1187, resp.; R 1 and wR 2 (all data): 0.1316 and 0.1353,
resp. Crystallographic data (excluding structure factors)
for the structure in this paper have been deposited with
the Cambridge Crystallographic Data Centre as supple-
mentary publication numbers CCDC 695426. Copies of
the data can be obtained, free of charge, on application to
CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax:
+44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk]

Cytotoxicity assay

The cytotoxicities of the isolates were evaluated using
the MTT method (Mosmann, 1983) with HL-60 cell
lines, and SRB method (Skehan et al., 1990) with A-549
cell lines.

RESULTS AND DISCUSSION

By analyzing their spectroscopic and physicochemical
properties, compounds 2-8 were identified as euphospocin
F, epieuphospocin B (Yamamura et al., 1989), euphospocin
A, euphospocin B (Yamamura et al., 1981), euphospocin
C (Yamamura et al., 1989), euphoheliosniod A (Zhang
and Guo, 2005), and euphorin (Yamamura et al., 1989)
(Fig. 1).

Compound 1 was obtained as colorless prism (MeOH)
### Table 1. $^1$H- and $^{13}$C-NMR data of 1, 2, 3, 5 and 6 in CDCl$_3$<sup>a</sup>

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_1$ (J in Hz)</th>
<th>$\delta_2$</th>
<th>$\delta_3$ (J in Hz)</th>
<th>$\delta_4$</th>
<th>$\delta_5$ (J in Hz)</th>
<th>$\delta_6$ (J in Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.66 (dd, 15.1, 6.6)</td>
<td>39.7t</td>
<td>3.07 (m), 2.25 (m)</td>
<td>43.5t</td>
<td>2.92 (dd, 14.8, 3.2)</td>
<td>1.46 (dd, 14.8, 9.6)</td>
</tr>
<tr>
<td>2</td>
<td>2.40 (m)</td>
<td>36.5d</td>
<td>2.26 (m)</td>
<td>39.2d</td>
<td>37.2d</td>
<td>2.45 (m)</td>
</tr>
<tr>
<td>3</td>
<td>4.78 (dd, 7.8, 7.3)</td>
<td>82.8d</td>
<td>5.19 (dd, 7.0, 2.3)</td>
<td>83.4d</td>
<td>5.00 (dd, 7.7, 5.1)</td>
<td>82.5d</td>
</tr>
<tr>
<td>4</td>
<td>3.58 (dd, 11.0, 7.8)</td>
<td>43.4d</td>
<td>3.44 (dd, 9.4, 7.0)</td>
<td>45.5d</td>
<td>3.32 (dd, 9.3, 7.7)</td>
<td>45.0d</td>
</tr>
<tr>
<td>5</td>
<td>5.73 (d, 11.5)</td>
<td>121.2d</td>
<td>5.70 (dd, 9.4, 11.9)</td>
<td>119.3d</td>
<td>5.67 (dd, 9.3)</td>
<td>122.4d</td>
</tr>
<tr>
<td>6</td>
<td>/</td>
<td>132.5s</td>
<td>/</td>
<td>139.9s</td>
<td>/</td>
<td>135.5s</td>
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<tr>
<td>7</td>
<td>4.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.0d</td>
<td>5.26 (dd, 11.1, 4.9)</td>
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<td>5.08 (dd, 9.6, 3.2)</td>
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<tr>
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<td>1.92 (2H, m)</td>
<td>32.2t</td>
<td>3.10 (dd, 15.1, 11.1)</td>
<td>42.5t</td>
<td>2.93 (dd, 14.1, 2.7)</td>
<td>39.8t</td>
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<tr>
<td>9</td>
<td>1.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.8d</td>
<td>/</td>
<td>206.6s</td>
<td>/</td>
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<td>/</td>
<td>50.3s</td>
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<tr>
<td>11</td>
<td>4.96 (d, 15.1)</td>
<td>135.9d</td>
<td>5.53 (d, 15.5)</td>
<td>136.3d</td>
<td>5.25 (d, 15.7)</td>
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<td>12</td>
<td>5.23 (d, 15.6, 9.1)</td>
<td>129.2d</td>
<td>5.03 (dd, 15.5, 9.7)</td>
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<td>5.34 (dd, 15.7, 7.7)</td>
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<td>2.30 (m)</td>
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<td>51.4d</td>
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<tr>
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<td>73.9d</td>
<td>/</td>
<td>211.6s</td>
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<tr>
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<td>/</td>
<td>92.1s</td>
<td>/</td>
<td>95.9s</td>
<td>/</td>
<td>93.0s</td>
</tr>
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<td>16</td>
<td>1.07 (3H, d, 7.0)</td>
<td>17.0q</td>
<td>1.14 (3H, d, 6.8)</td>
<td>18.8q</td>
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<td>17.9q</td>
</tr>
<tr>
<td>17</td>
<td>1.67 (3H, d, 0.9)</td>
<td>15.7q</td>
<td>1.67 (3H, d, 1.3)</td>
<td>18.7q</td>
<td>1.73 (H, d, 0.7)</td>
<td>17.4q</td>
</tr>
<tr>
<td>18</td>
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<td>22.5q</td>
<td>1.10 (3H, s)</td>
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<td>1.12 (3H, s)</td>
<td>24.5q</td>
</tr>
<tr>
<td>19</td>
<td>0.88 (3H, s)</td>
<td>20.1q</td>
<td>1.22 (3H, s)</td>
<td>20.1q</td>
<td>1.18 (3H, s)</td>
<td>22.6q</td>
</tr>
<tr>
<td>20</td>
<td>0.92 (3H, d, 6.8)</td>
<td>21.2q</td>
<td>1.22 (3H, d, 6.4)</td>
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<td>1.12 (3H, d, 6.4)</td>
<td>19.4q</td>
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<tr>
<td>3-OBez-1'</td>
<td>/</td>
<td>166.1s</td>
<td>/</td>
<td>165.2s</td>
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<td>165.7s</td>
</tr>
<tr>
<td>Bz(2')</td>
<td>/</td>
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<td>/</td>
<td>130.5s</td>
<td>/</td>
<td>130.4s</td>
</tr>
<tr>
<td>Bz(3', 7)</td>
<td>7.96 (2H, brd, 7.8)</td>
<td>129.3d</td>
<td>8.04 (2H, dd, 7.8, 1.2)</td>
<td>129.5d</td>
<td>7.95 (2H, brd, 7.7)</td>
<td>129.4d</td>
</tr>
<tr>
<td>Bz(4', 6)</td>
<td>7.41 (2H, dd, 7.8, 7.3)</td>
<td>128.3d</td>
<td>7.46 (2H, t, 7.8)</td>
<td>128.4d</td>
<td>7.41 (2H, t, 7.7)</td>
<td>128.2d</td>
</tr>
<tr>
<td>Bz(5')</td>
<td>7.53 (t, 7.3)</td>
<td>132.9d</td>
<td>7.56 (dd, 7.8, 1.4)</td>
<td>132.9d</td>
<td>7.53 (t, 7.7)</td>
<td>132.8d</td>
</tr>
<tr>
<td>7-OCCOCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.53 (3H, s)</td>
<td>20.5q</td>
<td>1.29 (3H, s)</td>
<td>19.0q</td>
<td>1.52 (3H, s)</td>
<td>20.4q</td>
</tr>
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<td>/</td>
<td>169.3s</td>
<td>/</td>
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<td>9-OCCOCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.98 (3H, s)</td>
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<tr>
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<td>21.3q</td>
<td>/</td>
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<td>21.1q</td>
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<td>169.9s</td>
<td>/</td>
<td>169.7s</td>
<td>/</td>
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<td>15-OCCOCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>2.29 (3H, s)</td>
<td>22.7q</td>
<td>2.36 (3H, s)</td>
<td>21.9q</td>
<td>2.16 (3H, s)</td>
<td>21.6q</td>
</tr>
<tr>
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<td>/</td>
<td>170.4s</td>
<td>170.8s</td>
<td>170.0s</td>
<td>170.0s</td>
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</tbody>
</table>

<sup>a</sup> The overlap signals.

with the specific rotation of $[\alpha]_{D}^{20} = -108.2^\circ$ (c 0.3, CHCl$_3$). Its molecular formula was established as C$_{35}$H$_{46}$O$_{10}$ by the positive HRESI-MS quasi-molecular ion at $m/z$ 649.3008 ([M + Na]$^+$, calc. 649.2989). IR absorption band at 1736 cm$^{-1}$ showed the existence of ester carbonyl group. Apart from substitution of an oxygenated methylene for a carbonyl group and an additional acetoxyl group, its 1D NMR data were similar to those of compound 3 (Table I), suggesting compound 1 as a benzoyloxy-substituted jatrophane diterpenoid tetraacetaete (Yamamura et al., 1989). $^1$H-$^1$H COSY and HMBC correlations allowed the construction of four structural moieties a-d (Fig. 2). The key HMBC correlations from H-17 to C-5, C-6, and C-7, together with the correlations between H-4 and C-6, and between H-5 and C-7 showed that a and b was connected together through the structural moiety -C(CHOH) -. The connectivity of b with d via C-10 in c was supported by the HMBC correlations between H-19 with C-9 and C-11, and between H-12 with C-10. And the diterpenoid skeleton was constructed completely via the C$_{4}$=C$_{15}$ single bond between a and d by HMBC correlations between H-1 and H-14 with C-15, and between H-14 with C-4 (Table I and...
The linkage of benzoyloxy and three acetoxy groups to C-3, C-7, C-9, C-14 were confirmed by corresponding HMBC correlations, respectively. The final acetoxy group was fixed to C-15 (Fig. 2). The relative configuration of compound 1 was dissolved by X-ray crystal diffraction (Fig. 3). On account of the angular proton, H-4, was biogenetically β-oriented, i.e. S-configuration of C-4 (Shizuri et al., 1984; Yamamura et al., 1981; Yamamura et al., 1989), the absolute configuration of compound 1 was established as 3S,4S,5E,7R,9R,11E,13S,14R,15R (Fig. 1).

The cytotoxicities of compounds 1-8 were assayed using the HL-60 cells by MTT method, and A-549 cells by SRB method. And VP-16 (etoposide) was used as the positive control with IC$_{50}$ values of 0.04 and 0.63 µM, respectively. Compounds 1 and 2 exhibited cytotoxicity against HL-60 with IC$_{50}$ values of 2.7 and 9.0 µM, respectively, while compounds 3-8 were inactive (IC$_{50}$ > 100 µM).

ACKNOWLEDGEMENTS

This work was supported by the project of Chinese National Programs for High Technology Research and Development (No. 2007AA091502 and 2007AA09z447). The cytotoxic assay was performed at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The medical material was identified by Dr Yan Luo, College of Marine Life, Ocean University of China.

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