

## New Compounds from *Euphorbia helioscopia* and Absolute Configuration Determination by Computational Methods

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The whole plant of *Euphorbia helioscopia* is an important traditional Chinese medicine. From its BuOH soluble extract, one new lactam (**1**), three new terpenoids (**2-4**) including a new naturally occurring compound, and three known compounds were isolated. Their structures were identified by spectroscopic evidences. In particular, the absolute configurations of side chain of compounds **1** and **2** were determined using computational methods.

**Key Words:** *Euphorbia helioscopia*, Euphorbiaceae, Lactam, Terpenoids, Quantum calculations

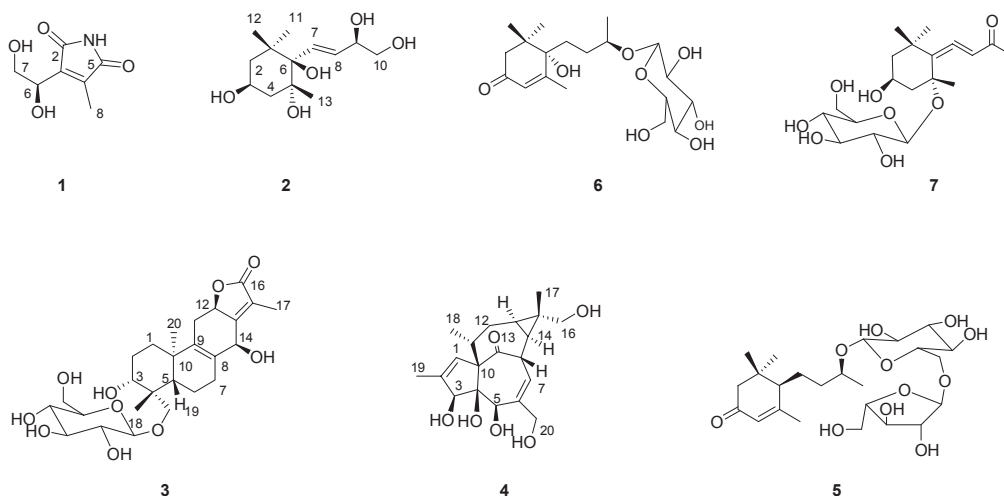
### Introduction

The plants of the genus *Euphorbia* (Euphorbiaceae) have been extensively investigated and considered to be a rich source of biologically active compounds.<sup>1</sup> *E. helioscopia* L. is a traditional Chinese medicine widely distributed in China and has been used for the treatment of malaria, bacillary dysentery, and osteomyelitis.<sup>2</sup> Previous reports on this plant mainly focused on diterpenoids, and up to now, almost 40 diterpenoids have been isolated.<sup>3-9</sup> During our investigation on *E. helioscopia*, seven compounds were isolated, 4 of which were new ones (Figure 1). Herein, we describe their isolation and structure elucidation.

### Results and Discussion

Compound **1** was determined to be C<sub>7</sub>H<sub>9</sub>NO<sub>4</sub> by its HRE-SIMS. The <sup>13</sup>C NMR spectrum exhibited one methyl, one oxy-

genated methylene, one oxygenated methine, two carbonyl groups, and two quaternary olefinic carbons. Two signals at  $\delta$  4.62 and  $\delta$  3.67/3.63 in the <sup>1</sup>H NMR spectrum corresponded to H-6 and H-7. Due to the scarcity of <sup>1</sup>H-<sup>1</sup>H correlations, the structure of **1** was mainly assembled by HMBC correlations. The HMBC observations of H-8/C-5, C-4 and C-3, H-6/C-4, C-3, C-2 and C-7, H-7/C-3 and C-6 deduced the partial structure of **1** to be C-2 to C-8. Besides two carbonyls, one double bond, one additional degree of unsaturation of **1** requires a ring constructed by C-2 and C-5 via NH group. The absolute stereochemistry of **1** was assigned by quantum calculations. For (*R*)-isomer, its det(D) of matrix was predicted as -10.8, the recorded optical rotation (OR) was -21.5. The  $k_0$  for **1** was 2.0, the predicted  $k_0$  was positive for chiral secondary alcohol. These exhibited that the absolute configuration at C-6 of **1** is *R*. This conclusion was further confirmed using DFT methods.<sup>10</sup> For example, *S*-isomer was used in OR computations in the gas phase, the



**Figure 1.** The structures of compounds **1-7**.

<sup>a</sup>These authors contributed equally to this paper.

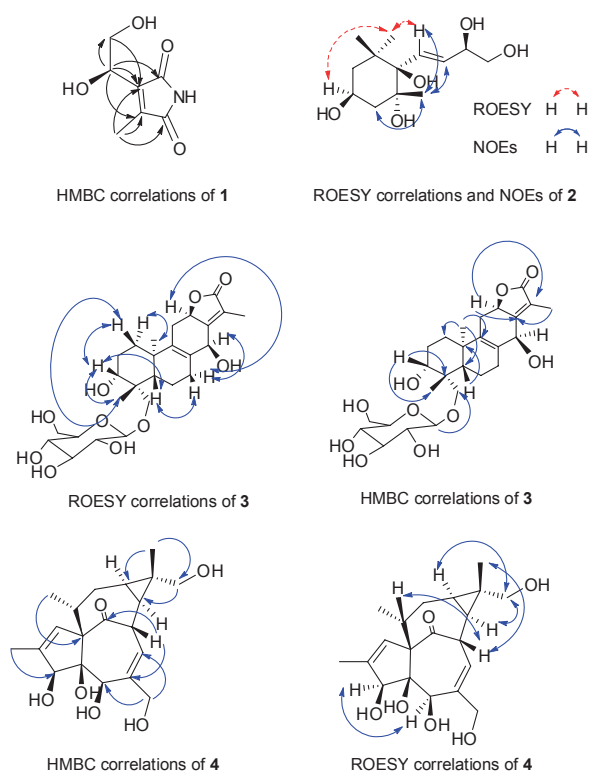
**Table 1.** NMR data for **1**<sup>a</sup> and **2**<sup>a</sup>

No	<b>1</b>		<b>2</b>	
	<sup>13</sup> C <sup>b</sup>	<sup>1</sup> H <sup>c</sup>	<sup>13</sup> C <sup>b</sup>	<sup>1</sup> H <sup>c</sup>
1			40.6	
2	173.7		46.3	1.64 (t, 12.1) 1.44 (ddd, 12.1, 4.1, 1.8)
3	140.2		65.2	4.04 (m)
4	141.9		45.7	1.75 (m)
5	174.4		77.8	
6	68.6	4.62 (t-like)	79.1	
7	65.6	3.67 (dd, 11.6, 4.8) 3.63 (dd, 11.6, 6.0)	133.7	6.17 (dd, 15.6, 1.2)
8	8.9	2.00 (s)	131.5	5.74 (dd, 15.6, 6.0)
9			74.6	4.21 (m)
10			67.7	3.51 (dd, 11.2, 4.5) 3.48 (dd, 11.2, 7.2)
11			26.2	1.20 (s)
12			27.5	0.83 (s)
13			27.2	1.13 (s)

<sup>a</sup>In CD<sub>3</sub>OD; <sup>b</sup>measured at 100 MHz; <sup>c</sup>measured at 400 MHz.

OR value was +81.2 without the consideration of water in solvent involved in the 1,2-diol structures. Therefore, the structure of **1** was identified (*R*)-3-(1,2-dihydroxyethyl)-4-methyl-1*H*-pyrrole-2,5-dione, namely heliolactam.

The molecular formula of **2** was determined as C<sub>13</sub>H<sub>24</sub>O<sub>5</sub> by its HRESIMS. The <sup>13</sup>C NMR data (Table 1) showed three methyl, three methylene, four methine (including two olefinic ones), and three quaternary carbons, suggesting **2** to be a megastigmane-type norsesquiterpenoid and the planar structure resembling the aglycone of kowiionoside.<sup>11</sup> The only difference was that C-10 of **2** was oxygenated which resulted in a downfield shift of C-10 at δ 67.7. The relative stereochemistry of **2** was determined by ROESY observations (Figure 2). ROESY correlations of H-11/H-3 and H-7 implied that OH-3 and OH-6 are both β. NOE enhancements of H-4, H-7, and H-8 in combination of the absence of that for H-3 when irradiating H-13 indicated that OH-5 is α-form. The *trans*-relationship of double bond was assigned according to the coupling constant of H-7 (*J* = 15.6 Hz). The absolute configuration of C-9 was determined by quantum calculations. The OR values were calculated using DFT methods.<sup>10</sup> Stable conformations were searched and the low energy conformations were optimized at the B3LYP/6-31G (d) level.<sup>12</sup> The conformations with relative energy from 0 - 2.5 kcal/mol were used in OR computations at the b3LYP/auccpVDZ level. The computed OR value for *S*-configuration at C-9 of **2** was +113 in the gas phase. The experimental OR value was -32.8. The two magnitudes had big differences and the OR signs were reversed. This exhibited that the stereogenic center is not *S*-configuration. OR computations for **2** with (*R*)-configuration at C-9 were performed using the same method as above. The calculated OR value was -12.0 in the gas phase. This value is close to the experimental -32.8, and the OR sign

**Figure 2.** Important HMBC and ROESY correlations for **1-4**.

agreed with the experimental one. Thus, the absolute configuration was predicted as *R* at C-9. The structure of **2** was therefore elucidated as (1*R*\*,2*R*\*4*S*\*)-1-((*R,E*)-3,4-dihydroxybut-1-enyl)-2,6,6-trimethylcyclohexane-1,2,4-triol, namely euphorheliosin A.

Compound **3** had the molecular formula C<sub>26</sub>H<sub>38</sub>O<sub>10</sub> deduced from its HRESIMS. The IR absorptions at 1738 and 1681 cm<sup>-1</sup> in combination with the <sup>13</sup>C NMR signals at δ 177.4, 163.7, and 121.7 (Table 2) revealed the presence of an α,β-unsaturated γ-lactone. Except for a glucosyl moiety, the <sup>13</sup>C NMR spectrum of **3** indicated an abietane diterpene characteristic of an α,β-unsaturated γ-lactone. Comparing with phlogacanthoside A,<sup>13</sup> the only difference for their planar structure was that **4** bears a hydroxyl at C-3, which was further supported by the HMBC responses of H-3/C-2, C-4, C-5, C-18, and C-19 (Figure 2). The relative stereochemistry of the backbone of **3** was determined by ROESY experiments: H-20/H-1α (δ 1.82), H-3/H-18 and H-1β (δ 1.22), H-5/H-7β (δ 2.42), H-7α (δ 2.02)/H-14 and H-12, assigning the relative configuration of the aglycone of **1** as shown. HMBC correlation of anomeric proton with C-19 confirmed the position of sugar moiety. Acid hydrolysis of **3** afforded D-glucose indicated by TLC comparison with authentic sample and its positive optical rotation. The glucose had a β-linkage with aglycone indicated by the coupling constant of anomeric proton (*J* = 7.6 Hz). **3** was determined as (3*R*\*,4*R*\*,4*aS*\*,7*S*\*,10*aR*\*,11*bR*\*)-3,7-dihydroxy-4,8,11*b*-trimethyl-4-(((2*R*\*,3*R*\*,4*S*\*,5*S*\*,6*R*\*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)methyl)-1,2,3,4,4*a*,5,6,10*a*,11,11*b*,-decahydrophenanthro[3,2-*b*]furan-9(7*H*)-one, namely euphorheliosin B.

Table 2. NMR data for **3**<sup>a</sup> and **4**<sup>b</sup>

No	<b>3</b>		<b>4</b>	
	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H
1	35.5	1.82 (m) 1.22 (m)	129.3	5.8 (s)
2	28.5	1.86 (m) 1.75 (m)	141.3	
3	80.1	3.21 (overlap)	80.7	4.35 (s)
4	43.6		86.0	
5	53.2	1.22 (overlap)	75.1	3.64 (s)
6	20.3	1.92 (m) 1.76 (m)	144.0	
7	30.5	2.42 (m) 2.02 (m)	124.3	6.0 (d, 5.0)
8	131.2		44.4	4.32 (overlap)
9	138.2		210.6	
10	39.2		74.1	
11	33.5	2.92 (dd, 14.7, 6.7) 1.84 (overlap)	40.5	2.48 (m)
12	79.7	4.82 (t, 8.0)	31.6	2.41 (ddd, 15.5, 9.0, 2.7) 1.74 (dt, 15.5, 6.0)
13	163.7		21.4	0.82 (m)
14	70.8	4.99 (s)	21.4	1.00 (dd, 12.0, 8.8)
15	121.7		31.3	
16	177.4		72.5	3.26 (s)
17	9.1	2.01 (s)	11.6	1.18 (s)
18	23.9	1.24 (s)	17.7	0.96 (d, 7.0)
19	72.4	4.23 (d, 10.0) 3.59 (d, 10.0)	15.5	1.82 (s)
20	19.2	1.15 (s)	65.6	4.11 (d, 13.7) 4.04 (d, 13.7)
Glc-1	104.9	4.23 (d, 7.6)		
2	75.1	3.21 (m)		
3	77.9	3.29 (m)		
4	71.6	3.26 (m)		
5	78.3	3.37 (m)		
6	62.7	3.89 (dd, 11.5, 1.2) 3.69 (dd, 11.5, 4.8)		

<sup>a</sup>Measured at 100 MHz for <sup>13</sup>C and 400 MHz for <sup>1</sup>H; <sup>b</sup>measured at 125 MHz for <sup>13</sup>C and 500 MHz for <sup>1</sup>H.

The molecular formula of **4** was deduced as C<sub>20</sub>H<sub>28</sub>O<sub>6</sub> by its HRESIMS. The <sup>13</sup>C NMR spectrum revealed three methyl, three methylene, eight methin, and six quaternary carbons. The planar structure of **4** was assembled mainly by the COSY and HMBC correlations (Figure 2). Comparing to ingenol,<sup>14</sup> the only difference was that C-16 was oxygenated in **4**, corresponding to a downfield shift for C-16 (δ 72.5). The relative configuration of **4** was determined by ROESY experiments, which showed interactions of H-8/H-11 and H-17, H-16/H-13 and H-14, and H-3/H-5, assigning the relative configurations of C-3, C-4, C-5, C-8, C-11, C-13, C-14, and C-15. This conclusion was identical with ingenol previously determined by X-ray diffraction. Accord-

dingly, **4** was identified as 16-hydroxyingenol. Compound **4** was characterized previously by base catalysed transesterification from ingenol.<sup>15</sup> However, it was firstly isolated as a new naturally occurring compound. Further, the <sup>1</sup>H, <sup>13</sup>C NMR data of **4** were firstly unambiguously assigned using 2D NMR techniques.

Known compounds were identified as leaaside (**5**),<sup>16</sup> roseoside II (**6**),<sup>17</sup> and citroside A (**7**)<sup>18</sup> by comparison of their spectroscopic data with literature values. This study provides a new insight into the chemical profiling of this plant.

### Experimental Section

**General procedures.** Column chromatography (CC) was performed on silica gel (200 - 300 mesh; Qingdao Marine Chemical Inc., China), on C<sub>18</sub> reverse-phase silica gel (40 - 60 μm; Daiso Co., Japan), MCI gel CHP 20P (75 - 150 μm, Tokyo, Japan) and on Sephadex LH-20 (Amersham Pharmacia, Sweden). Semi-preparative HPLC was carried out on an Agilent 1100 liquid chromatography with a Zorbax SB-C<sub>18</sub> column (9.4 × 250 mm, i.d.). UV Spectra were obtained on a Shimadzu double-beam 210A spectrometer, λ<sub>max</sub> in nm. Optical rotations were recorded on a Horiba SEPA-300 polarimeter. IR Spectrum was determined by a Tensor 27 spectrometer, with KBr pellets; in cm<sup>-1</sup>. NMR Spectra were measured on a Bruker AV-400 or a DRX-500 spectrometer, with TMS as an internal standard. FABMS was determined on a VG Autospec-3000 spectrometer. ESIMS and HRESIMS were collected by a API QSTAR Pulsar 1 spectrometer.

**Plant material.** The whole plants of *E. helioscopia* were purchased from Kunming Juhucun market, Kunming, Yunnan Province, China, in July 2008, and authenticated by Mr. B. Qiu. A voucher specimen (CHYX-0151) was deposited at the State Key Laboratory of Photochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

**Extraction and isolation.** The dried whole plant powders of *E. helioscopia* (15 kg) were extracted with MeOH under reflux (3 × 30 L). The extracts were combined and concentrated in vacuo to yield a dark green residue, which was suspended in water followed by successive partition with petroleum ether (3 × 3 L), EtOAc (3 × 3 L), and *n*-BuOH (3 × 3 L). The *n*-BuOH extract (80 g) was separated by a silica gel CC (8.5 × 120 cm, 200 - 300 mesh, 1.5 kg) eluted with a gradient of CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O to afford four fractions (A-D). Fr. A (5.22 g) was subjected to MCI gel CHP 20P eluted with a gradient aqueous MeOH (30 - 60%) to yield three subfractions (AI-AIII). Fr. AI (1.2 g) was gel filtrated on Sephadex LH-20 to obtain **4** (25 mg). Fr. AIII (800 mg) was chromatographed on Sephadex LH-20 (MeOH) to yield **2** (3 mg). Fr. B (4.3 g) was subjected to MCI gel CHP 20P with gradient aqueous MeOH (30 - 60%) as eluents to produce two portions (BI-BII). Fr. BI (1.1 g) was submitted to gel filtration on Sephadex LH-20 (MeOH) to obtain **6** (18 mg). Fr. BII was purified on Sephadex LH-20 (MeOH) to produce **7** (14 mg). Fr. C (5.8 g) was fractionated by MCI gel CHP 20P eluted with MeOH/H<sub>2</sub>O (20 - 70%) to afford two portions (CI-CII). Fr. CI (2.0 g) was further separated on Sephadex LH-20 eluting with MeOH to get CI-I (50 mg), CI-II (300 mg), and



CI-III (500 mg). Fr. CI-I was purified by semi-preparative HPLC (MeOH/H<sub>2</sub>O, 40:60) to yield **3** (6 mg). Fr. CII (1.3 g) was passed through Sephadex LH-20 eluting with MeOH to afford CII-I (400 mg), which was subjected to C<sub>18</sub> gel (MeOH/H<sub>2</sub>O, 30%) to yield **1** (15 mg). Fr. D (3.1 g) was divided into three fractions (DI-DIII) by MCI gel CHP 20P chromatography using gradient MeOH/H<sub>2</sub>O (20 - 70%) as mobile phase. Fr. DII (1.1 g) was subjected to Sephadex LH-20 (MeOH) to produce DII-I (200 mg), which was further purified by semi-preparative HPLC with MeOH/H<sub>2</sub>O (30%) as mobile phase to afford **5** (4 mg).

**(R)-3-(1,2-Dihydroxyethyl)-4-methyl-1H-pyrrole-2,5-dione (1):** Colorless oils;  $R_f$  = 0.46, silica gel GF<sub>254</sub>, CHCl<sub>3</sub>/MeOH (4:1);  $[\alpha]_D^{24}$  = -21.5 ( $c$  = 0.3, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 222.0 (4.03), 196.8 (3.81); IR (KBr)  $\nu_{\max}$  3411, 1712, 1356, 740 cm<sup>-1</sup>; <sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) data see Table 1. ESIMS (negative) 170 [M-H]<sup>-</sup>; HRESIMS (positive) 194.0431 ([M+Na]<sup>+</sup>, calcd. for C<sub>7</sub>H<sub>9</sub>NO<sub>4</sub>Na, 194.0429).

**(1R\*,2R\*,4S\*)-1-((R,E)-3,4-Dihydroxybut-1-enyl)-2,6,6-trimethylcyclohexane-1,2,4-triol (2):** Colorless oils;  $R_f$  = 0.50, silica gel GF<sub>254</sub>, CHCl<sub>3</sub>/MeOH (4:1);  $[\alpha]_D^{23}$  = -32.8 ( $c$  = 0.24, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 202.2 (3.63); IR (KBr)  $\nu_{\max}$  3406, 3260, 2954, 2926, 2871, 1628, 1374, 1077 cm<sup>-1</sup>; <sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) data see Table 1; ESIMS (negative) 295 [M+Cl]<sup>-</sup>; HRESIMS (negative) 295.1316 ([M+Cl]<sup>-</sup>, calcd. for C<sub>13</sub>H<sub>24</sub>O<sub>5</sub>Cl 295.1312).

**(3R\*,4R\*,4aS\*,7S\*,10aR\*,11bR\*)-3,7-Dihydroxy-4,8,11b-trimethyl-4-(((2R\*,3R\*,4S\*,5S\*,6R\*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy)methyl)-1,2,3,4,4a,5,6,10a,11,11b-decahydrophenanthro[3,2-b]furan-9(7H)-one (3):** Colorless oils;  $R_f$  = 0.42, silica gel GF<sub>254</sub>, CHCl<sub>3</sub>/MeOH (4:1);  $[\alpha]_D^{24}$  = -147.4 ( $c$  = 0.4, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 219.2 (4.14); IR (KBr)  $\nu_{\max}$  3422, 2926, 1738, 1681, 1080 cm<sup>-1</sup>; <sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) data see Table 2; FABMS (negative) 509 [M-H]<sup>-</sup>; HRESIMS (negative) 545.2136 ([M+Cl]<sup>-</sup> [M+Cl]<sup>-</sup> calcd. for C<sub>26</sub>H<sub>38</sub>O<sub>10</sub>Cl 545.2153).

**16-Hydroxyingenol (4):** Yellowish oils;  $R_f$  = 0.55, silica gel GF<sub>254</sub>, CHCl<sub>3</sub>/MeOH (5:1);  $[\alpha]_D^{24}$  = -20.0 ( $c$  = 0.45, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 203.2 (3.87); IR (KBr)  $\nu_{\max}$  3422, 2874, 1708, 1015 cm<sup>-1</sup>; <sup>1</sup>H (500 MHz) and <sup>13</sup>C NMR (125 MHz) data see Table 2; FABMS (negative) 363 [M-H]<sup>-</sup>; HRESIMS (negative) 363.1802 ([M-H]<sup>-</sup> calcd. for C<sub>20</sub>H<sub>27</sub>O<sub>6</sub> 363.1807).

**Acid hydrolysis.** A solution of **3** (2 mg) in 2 M HCl (4 mL) was heated in a water bath at 70 °C for 6 h. After cooling, the mixture was neutralized with NaHCO<sub>3</sub> and extracted with CHCl<sub>3</sub>. TLC comparison with authentic sample revealed the presence of glucose in the water layer. The D-form of glucose was determined by its positive optical rotation in water ( $[\alpha]_D^{24.0}$  = +38.6 ( $c$  = 0.01, H<sub>2</sub>O)) for **3** and ( $[\alpha]_D^{24.0}$  = +50.4 ( $c$  = 0.53, H<sub>2</sub>O)) for the authentic sample.

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**Supporting Information.** NMR and MS data of **1-4** are available on line.

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