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

Jiangbo He,[†,§,a] Hua-Jie Zhu,[†,a] Gui-Fen Luo,[†] Guang-Ming Liu,[§] Hao Chen,[‡] Yan Li,[†] Shaopeng Chen,[‡] Xin Lu,[‡] Guochun Zhou,[‡] and Yong-Xian Cheng[†,*]

[†]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China. *E-mail: yxcheng@mail.kib.ac.cn*
[‡]*Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China*
[§]*College of Pharmaceutical Sciences, Dali University, Dali 671002, China*
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The whole plant of *Euphorbia helioscopia* is an important traditional Chinese medicine. Fom 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.[1] *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.[2] Previous reports on this plant mainly focused on diterpenoids, and up to now, almost 40 diterpenoids have been isolated.[3-9] 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 $C_7H_9NO_4$ by its HRESIMS. The ^{13}C NMR spectrum exhibited one methyl, one oxygenated methylene, one oxygenated methine, two carbonyl groups, and two quaternary olefinic carbons. Two signals at δ 4.62 and δ 3.67/3.63 in the ^1H NMR spectrum corresponded to H-6 and H-7. Due to the scarcity of ^1H-^1H 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.[10] For example, *S*-isomer was used in OR computations in the gas phase, the

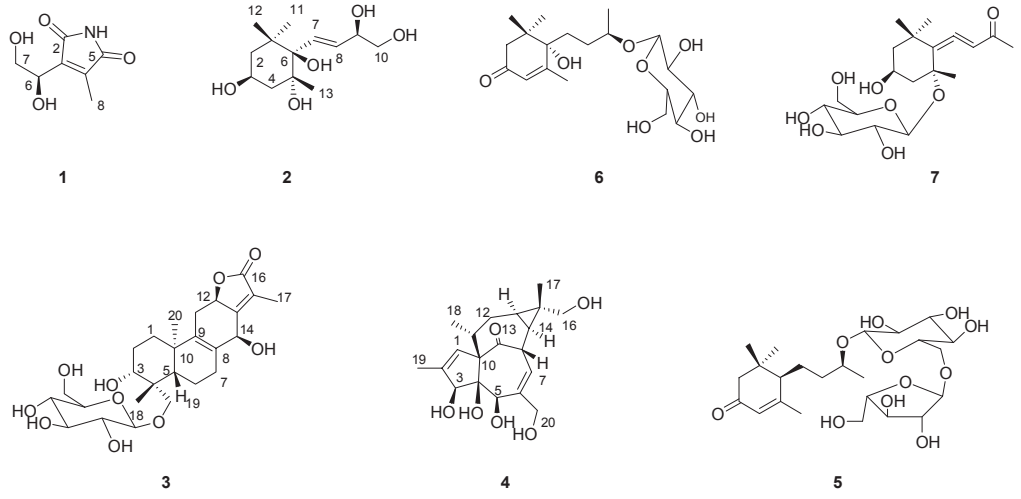

Figure 1. The structures of compounds **1**-**7**.

[a]These authors contributed equally to this paper.

Table 1. NMR data for **1**[a] and **2**[a]

No	**1**		**2**	
	^{13}C[b]	^{1}H[c]	^{13}C[b]	^{1}H[c]
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)

[a]In CD_3OD; [b]measured at 100 MHz; [c]measured at 400 MHz.

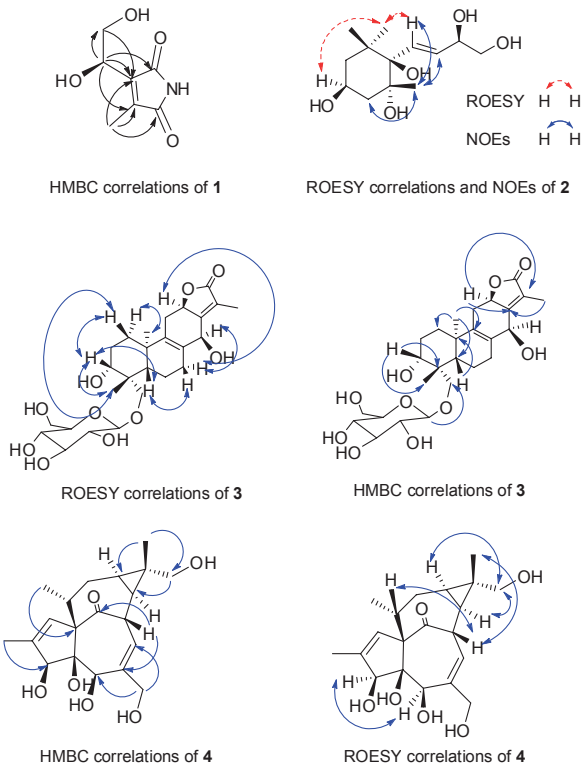

Figure 2. Important HMBC and ROESYcorrelations for **1-4**.

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 $C_{13}H_{24}O_5$ by its HRESIMS. The ^{13}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.[11] 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.[10] Stable conformations were searched and the low energy conformations were optimized at the B3LYP/6-31G (d) level.[12] The conformations with relative energy from 0 - 2.5 kcal/mol were used in OR computations at the b3LYP/augcc-pVDZ 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 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 $C_{26}H_{38}O_{10}$ deduced from its HRESIMS. The IR absorptions at 1738 and 1681 cm^{-1} in combination with the ^{13}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 ^{13}C NMR spectrum of **3** indicated an abietane diterpene characteristic of an α,β-unsaturated γ-lactone. Comparing with phlogacanthoside A,[13] 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**,4a*S**,7*S**,10a*R**,11b*R**)-3,7-dihydroxy-4,8,11b-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)-1,2,3,4,4a,5,6,10a,11,11b,-decahydrophenanthro[3,2-b]furan-9(7*H*)-one, namely euphorheliosin B.

Table 2. NMR data for **3**[a] and **4**[b]

No	**3**		**4**	
	^{13}C	^{1}H	^{13}C	^{1}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)		

[a]Measured at 100 MHz for ^{13}C and 400 MHz for ^{1}H; [b]measured at 125 MHz for ^{13}C and 500 MHz for ^{1}H.

The molecular formula of **4** was deduced as $C_{20}H_{28}O_6$ by its HRESIMS. The ^{13}C NMR spectrum revealed three methyl, three methylene, eight methin, and six quarternary carbons. The planar structure of **4** was assembled mainly by the COSY and HMBC correlations (Figure 2). Comparing to ingenol,[14] 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. Accordingly, **4** was identified as 16-hydroxyingenol. Compound **4** was characterized previously by base catalysed transesterification from ingenol.[15] However, it was firstly isolated as a new naturally occurring compound. Further, the ^{1}H, ^{13}C NMR data of **4** were firstly unambiguously assigned using 2D NMR techniques.

Known compounds were identified as leeaoside (**5**),[16] roseoside II (**6**),[17] and citroside A (**7**) [18] 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_{18} 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_{18} column (9.4 × 250 mm, i.d.). UV Spectra were obtained on a Shimadzu double-beam 210A spectrometer, λ_{max} 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^{-1}. 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. heliscopia* were purchased from Kunming Juhuacun 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. heliscopia* (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 $CHCl_3$/MeOH/H_2O 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. AII (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_2O (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_2O, 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_{18} gel (MeOH/H_2O, 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_2O (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_2O (30%) as mobile phase to afford **5** (4 mg).

(*R*)-3-(1,2-Dihydroxyethyl)-4-methyl-1*H*-pyrrole-2,5-dione (1): Colorless oils; R_f = 0.46, silica gel GF_{254}, $CHCl_3$/MeOH (4:1); $[\alpha]_D^{24}$ = −21.5 (*c* = 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 222.0 (4.03), 196.8 (3.81); IR (KBr) ν_{max} 3411, 1712, 1356, 740 cm^{-1}. ^1H (400 MHz) and ^{13}C NMR (100 MHz) data see Table 1. ESIMS (negative) 170 $[M-H]^-$; HRESIMS (positive) 194.0431 ($[M+Na]^+$, calcd. for $C_7H_9NO_4Na$,194.0429).

(1*R,2*R***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_{254}, $CHCl_3$/MeOH (4:1); $[\alpha]_D^{23}$ = −32.8 (*c* = 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 202.2 (3.63); IR (KBr) ν_{max} 3406, 3260, 2954, 2926, 2871, 1628, 1374, 1077 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data see Table 1; ESIMS (negative) 295 $[M+Cl]^-$; HRESIMS (negative) 295.1316 ($[M+Cl]^-$, calcd. for $C_{13}H_{24}O_5Cl$ 295.1312).

(3*R,4*R**,4a*S**,7*S**,10a*R**,11b*R**)-3,7-Dihydroxy-4,8,11b-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)-1,2,3,4,4a,5,6,10a,11,11b-decahydrophenanthro[3,2-b]furan-9(7*H*)-one (3):** Colorless oils; R_f = 0.42, silica gel GF_{254}, $CHCl_3$/MeOH (4:1); $[\alpha]_D^{24}$ = −147.4 (*c* = 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 219.2 (4.14); IR (KBr) ν_{max} 3422, 2926, 1738, 1681, 1080 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (100 MHz) data see Table 2; FABMS (negative) 509 $[M-H]^-$; HRESIMS (negative) 545.2136 ($[M+Cl]$ $[M+Cl]^-$ calcd. for $C_{26}H_{38}O_{10}Cl$ 545.2153).

16-Hydroxyingenol (4): Yellowish oils; R_f = 0.55, silica gel GF_{254}, $CHCl_3$/MeOH (5:1); $[\alpha]_D^{24}$ = −20.0 (*c* = 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 203.2 (3.87); IR (KBr) ν_{max} 3422, 2874, 1708, 1015 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data see Table 2; FABMS (negative) 363 $[M-H]^-$; HRESIMS (negative) 363.1802 ($[M-H]^-$ calcd. for $C_{20}H_{27}O_6$ 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 $NaHCO_3$ and extracted with $CHCl_3$. 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_2O)) for **3** and ($[\alpha]_D^{24.0}$ = +50.4 (*c* = 0.53, H_2O)) for the authentic sample.

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

References

1. Shi, Q. W.; Su, X. H.; Kiyota, H. *Chem. Rev*. **2008**, *108*, 4295.
2. Hua, Y. X.; Liu, S. F.; Yang, Z. Q. *Chinese Bencao*; Shanghai Science and Techology Press: Shanghai, 1999; p 782.
3. Lu, Z. Q.; Guan, S. H.; Li, X. N.; Chen, G. T.; Zhang, J. Q.; Huang, H. L.; Liu, X.; Guo, D. A. *J. Nat. Prod*. **2008**, *71*, 873.
4. Lu, Z. Q.; Yang, M.; Zhang, J. Q.; Chen, G. T.; Huang, H. L.; Guan, S. H.; Ma, C.; Liu, X.; Guo, D. A. *Phytochemistry* **2008**, *69*, 812.
5. Zhang, W.; Guo, Y. W. *Planta Med*. **2005**, *71*, 283.
6. Zhang, W.; Guo, Y. W. *Chem. Pharm. Bull*. **2006**, *54*, 1037.
7. Yamamura, S.; Shizuri, Y.; Kosemura, S.; Ohtsuka, J.; Tayama, T.; Ohba, S.; Ito, M.; Saito, Y.; Terada, Y. *Phtochemistry* **1989**, *28*, 3421.
8. Kosemura, S.; Shizuri, Y.; Yamamura, S. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 3112.
9. Yamamura, S.; Kosemura, S.; Ohba, S.; Ito, M.; Saito, Y. *Tetrahedron Lett.* **1981**, *22*, 5315.
10. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03 User's Reference*, Gaussian, Inc., Pittsburgh, PA, 2003, 15106.
11. Takeda, Y.; Okada, Y.; Masuda, T. *Chem. Pharm. Bull*. **2000**, *48*, 752.
12. a) Timmons, C.; Wipf, P. *J. Org. Chem*. **2008**, *73*, 9168. b) Chen, L. X.; Zhu, H. J.; Wang, R.; Zhou, K. L.; Jing, Y. K.; Qiu, F. *J. Nat. Prod*. **2008**, *71*, 852. c) Pu, J. X.; Huang, S. X.; Ren, J.; Xiao, W. L.; Li, L. M.; Li, R. T.; Li, L. B.; Liao, T. G.; Lou, L. G.; Zhu, H. J.; Sun, H. D. *J. Nat. Prod*. **2007**, *70*, 1706. d) Hua, Y.; Ren, J.; Chen, C. X.; Zhu, H. J. *Chem. Res. Chin. U*. **2007**, *23*, 592.
13. Yuan, X. H.; Li, B. G.; Zhang, X. Y. *J. Nat. Prod.* **2005**, *68*, 86.
14. Appendino, G.; Tron, G. C.; Cravotto, G. *J. Nat. Prod.* **1999**, *62*, 76.
15. Opferkuch, H. J.; Hecker, E. *Tetrahedron Lett.* **1974**, *3*, 261.
16. Kaewkrud, W.; Otsuka, H.; Somsak, R. *J. Nat. Med*. **2007**, *61*, 449.
17. Otsuka, H.; Yao, M.; Kamada, K. *Chem. Pharm. Bull*. **1995**, *43*, 754.
18. Umehara, K.; Hattori, I.; Miyase, T. *Chem. Pharm. Bull*. **1988**, *36*, 5004.