

Trijugin-Type Limonoids from the Leaves of *Cipadessa cinerascens*

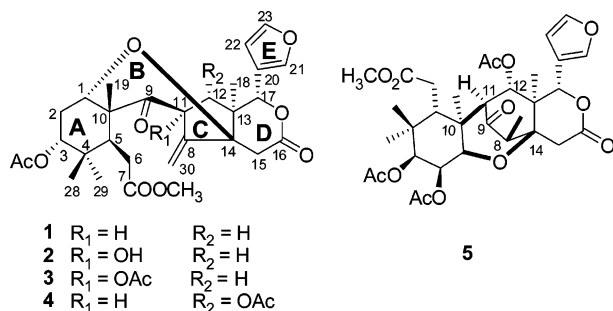
Ying-Tong Di,^{†,‡} Hong-Ping He,[†] Hai-Yang Liu,[†] Ping Yi,[†] Zhen Zhang,[§] Yan-Li Ren,[⊥] Jun-Song Wang,[†] Qian-Yun Sun,^{||} Fu-Mei Yang,^{||} Xin Fang,[†] Shun-Lin Li,[†] Hua-Jie Zhu,[†] and Xiao-Jiang Hao^{*,†}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China, Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China, College of Pharmaceutical Sciences, Dali University, Dali 677100, People's Republic of China, College of Living Creature Science and Technology, Hunan Agricultural University, Changsha 410128, Hunan, People's Republic of China, and Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, People's Republic of China

Received February 9, 2007

Four new trijugin-type limonoids, cipatrijugins A–D (**1**–**4**), together with the known cipadesin A (**5**), were isolated from the leaves of *Cipadessa cinerascens*, and their structures were elucidated on the basis of spectroscopic and computational methods. The ability of compounds **1**–**5** to inhibit the growth of the A549 and K562 tumor cell lines was evaluated.

Limonoids, produced mainly by plants in the families Rutaceae and Meliaceae, have attracted great interest due to their diverse structures and significant biological activities.^{1,2} *Cipadessa cinerascens* (Pell.) Hand.-Mazz. (Meliaceae) is a medicinal plant widely distributed in the southwest of mainland China. The leaves and roots of the plant have been used for the treatment of rheumatism, malaria, scalds, and skin itches.³ In previous phytochemical investigations, limonoids and other types of compounds have been isolated from species in the genus *Cipadessa*.^{4,5} The present study on the leaves of *C. cinerascens* led to the isolation of four new trijugin-type limonoids, cipatrijugins A–D (**1**–**4**), together with the known compound cipadesin A (**5**). To the best of our knowledge, this is the first report of trijugin-type limonoids from the genus *Cipadessa*.⁶



Cipatrijugin A (**1**) was obtained as a white powder. The molecular formula was determined to be C₂₉H₃₆O₉ from the [M + Na]⁺ ion peak at *m/z* 551.2248 in the HRESIMS. IR absorption bands at 1737, 1682, and 1644 cm⁻¹ revealed a ketonic carbonyl group (δ_C 210.2) and three ester carbonyl groups (δ_C 174.4, 170.7, and 168.8). The ¹H and ¹³C NMR spectra of **1** (Table 1) exhibited resonances for one acetyl group (δ_H 2.04, δ_C 20.8, 168.8), four quaternary methyls, one carbomethoxy, one exomethylene, one ketonic carbonyl, one ring-D lactone, one β -substituted furan, and four further oxygen-bearing carbons (three secondary and one quaternary). These signals together with four methylenes, three methines, and two quaternary carbon atoms accounted for all 29

Table 1. NMR Data of Cipatrijugin A (**1**) in CDCl₃ at 298 K

no.	δ_C	δ_H	no.	δ_C	δ_H
1	71.6	4.16 (1H, t, 2.5)	16	170.7	
2 α	29.6	2.19 (1H, dt, 16.0, 2.5)	17	79.3	6.41 (1H, s)
2 β		1.91 (1H, dt, 16.0, 2.5)			
3	74.8	4.79 (1H, t, 2.5))	18	17.5	0.78 (3H, s)
4	38.3		19	19.9	1.02 (3H, s)
5	37.9	2.98 (1H, t, 4.5)	20	121.9	
6 α	29.6	2.90 (1H, dd, 18.0, 4.5)	21	143.5	7.55 (1H, s)
6 β		2.27 (1H, dd, 18.0, 4.5)			
7	174.4		22	108.6	6.46 (1H, br s)
8	144.5		23	139.8	7.44 (1H, br s)
9	210.2		28	27.5	0.87 (3H, s)
10	55.2		29	22.9	0.95 (3H, s)
11	58.6	3.52 (1H, dd, 4.0, 10.0)	30a	114.1	5.37 (1H, br s)
			30b		5.17 (1H, br s)
12 α	35.7	1.70 (1H, dd, 14.0, 10.0)	Ac-3	20.8	2.04 (3H, s)
12 β		2.81 (1H, dd, 14.0, 4.0)			
13	46.0			168.8	
14	87.5		OCH ₃	51.7	3.66 (3H, s)
15	34.5	2.79 (2H, m)			

carbon atoms in **1**. The above-mentioned spectroscopic features and comparison to known compounds implied that **1** might have a partial structure similar to cipadesin A or trijugin.^{4b,6} The planar structure was confirmed by the HMBC NMR spectrum. HMBC correlations (Figure 1) H-17/C-12, C-13, and C-14 and H-30/C-8, C-11, and C-14 showed a five-membered ring C connected to ring D at C-13 and C-14. The connectivity between rings A and C was indicated by the three-bond correlations between H-1/C-14, Me-19/C-9, H-5/C-9, H₂-12/C-9, and H-9/C-10. Cross-peaks between H-3 and the acetyl carbonyl carbon signals in the HMBC spectrum were used to place the acetoxy group at C-3.

The relative configuration of **1** was constructed from analysis of molecular models, energy minimized using density functional theory (DFT) at the 3-21G* basis set level in Gaussian 03 overlaid with key correlations observed in the ROESY NMR spectrum (Figure 2A). The small coupling constant ($J = 2.5$ Hz) of H-3 with H₂-2 and cross-peaks between Me-3-OAc and H-17 in the ROESY spectrum indicated that the C-3 acetoxy group and H-17 are both in the axial orientation. Clear ROE correlations between H-5/H-17 and H-12 β /H-17 indicated that H-5 and H-12 β also have the same orientation, which were supported by calculated interatomic distances of approximately 2.5 Å. Cross-peaks between H-1/Me-19, H-1/H-30a, H-12 α /Me-18, and H-11/H-30b also were observed in the ROESY spectrum, suggesting that H-1, Me-18, Me-19, and H₂-30 are all in an α -orientation. Furthermore, the large coupling constant between H-9 and H-12 α ($J = 10.0$ Hz) suggested their

* Corresponding author. Tel: +86-871-5223263. Fax: +86-871-5219684. E-mail: haoxj@mail.kib.ac.cn.

[†] Kunming Institute of Botany.

[‡] Graduate School of Chinese Academy of Sciences.

[§] Dali University.

[⊥] Hunan Agricultural University.

^{||} Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

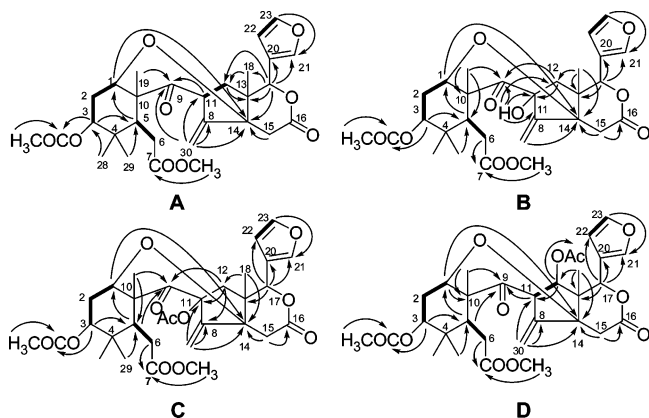


Figure 1. ^1H – ^1H COSY (→) and HMBC (↔) correlations of cipatrijugins A–D (1–4).

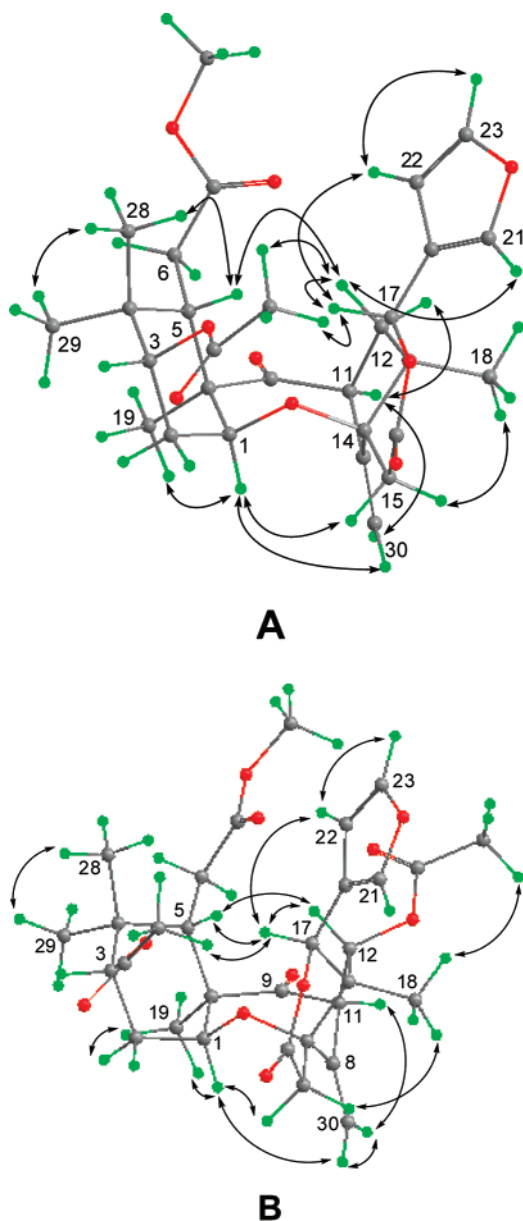


Figure 2. DFT-calculated energy-minimized models of cipatrijugin A (1) and D (4) illustrating the major ROESY correlations (↔) used to define the relative stereochemistry.

cis-diaxial orientation in the five-membered ring C, supported by a calculated distance of 2.3 Å from the molecular model. Moreover, the calculated result also suggested that ring A adopts a chair

conformation, ring B a twist-boat conformation, ring C an envelope conformation, and ring D a half-chair conformation. Therefore, the structure of **1**, a new trijugin-type limonoid, was elucidated as shown.

Cipatrijugin B (**2**) was obtained as a white powder, and a molecular formula of $\text{C}_{29}\text{H}_{36}\text{O}_{10}$ was deduced from the $[\text{M} + \text{Na}]^+$ ion peak at m/z 567.2206 in the HRESIMS. The ^1H and ^{13}C NMR data of **2** (Table 2) were closely related to those of **1**, except for the appearance of one oxygen-bearing quaternary carbon instead of a methine in **1**. The exchangeable sharp singlet at δ_{H} 4.73 (1H) in the ^1H NMR spectrum (removed on addition of D_2O) was ascribed to the proton of the hydroxy group attached to C-11, which was supported by HMBC correlations between the hydroxy proton (δ_{H} 4.73) and C-8 (δ_{C} 147.4), C-9 (δ_{C} 211.4), C-11 (δ_{C} 85.7), and C-12 (δ_{C} 46.6). ROESY correlations of H-17/H-12 β , H-17/H-5, and H-5/H-12 β indicated that the chemical bonds C-11–C-12 and C-9–C-11 occurred on the same side of the molecule, with the hydroxyl at C-11 on the other side. The remaining part of the structure and the relative configuration of **2** were similar to those of **1**, as determined from the HMBC (Figure 1B) and ROESY NMR spectra.

Cipatrijugin C (**3**) also was obtained as a white powder, and its molecular formula, $\text{C}_{31}\text{H}_{38}\text{O}_{11}$, was determined by the $[\text{M} + \text{Na}]^+$ ion peak at m/z 609.2293 in the HRESIMS. The ^1H and ^{13}C NMR data of **3** (Table 2) were closely related to those of **2**, except for the presence of acetyl signals (δ_{H} 2.01, δ_{C} 169.9, 20.9) in **3**. The ^{13}C NMR signal for C-11 occurred downfield about 6 ppm and those for C-8, C-9, and C-12 were shifted upfield by 3–8 ppm, indicating the presence of an acetate group at C-11, which was confirmed by HMBC correlations between H-12 and C-8, C-9, and C-11. The remaining structure and relative configuration of **3** were identical to those of **2**, as determined by HMBC (Figure 1C) and ROESY NMR experiments.

Cipatrijugin D (**4**) was obtained as a white powder. The ^1H and ^{13}C NMR spectroscopic data of **4** (Table 2) were analogous to those of **3**, except for the presence of two methine carbons, one of which was found to bear an oxygen atom, instead of the methylene carbon and an oxygen-bearing quaternary carbon as in **3**. The molecular formula $\text{C}_{31}\text{H}_{38}\text{O}_{11}$ of **4**, identical to that of **3**, suggested that both compounds have the same skeleton. Two acetoxy groups were located at C-3 and C-12 by the HMBC cross-peaks H-3 (δ_{H} 4.83)/C-3-OAc (δ_{C} 170.7) and H-12 (δ_{H} 5.56)/C-12-OAc (δ_{C} 168.8), respectively. The small coupling constant ($J = 3.0$ Hz) of H-11 with H-12 and ROESY correlations of H-12/H-17, H-12/H-5, H-17/H-5, and H-9/H-30a indicated a *trans* relationship between the C-12 acetoxy group and the C-9 substituent on the cyclopentane ring. Since the other HMBC (Figure 1D) and ROESY data were similar to those of **3**,⁸ the structure of cipatrijugin D (**4**) was elucidated as shown in Figure 2B.

The *in vitro* cytotoxic activities of cipatrijugins A–D (1–4) and cipadesin A (5) against the growth of two tumor cell lines (A549, human lung adenocarcinoma, and K562, human lymphocytic leukemia) were evaluated.⁹ However, all compounds tested were inactive against these two cancer cells (50% effective dose of clonal inhibition, $\text{ED}_{50} > 5 \mu\text{g/mL}$).

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer model 241 polarimeter. IR spectra were measured in a Bio-Rad FTS-135 spectrometer as KBr pellets. ^1H and 2D NMR spectra were measured on a Bruker DRX-500 instrument, while ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer. Chemical shifts are reported using residual CHCl_3 (δ_{H} 7.26 and δ_{C} 77.0) as internal standard. ESIMS and HRESIMS spectra were recorded using a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. Column chromatography was performed on silica gel (90–150 μm ; Qingdao Marine Chemical Plant, Qingdao, People's Republic of China), Sephadex LH-20 (40–70 μm , Amersham

Table 2. NMR Data of Cipatrijugins B–D (2–4) at 298 K

position	2^a		3^b		4^a	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	4.00 (1H, t, 3.0)	71.5	3.97 (1H, t, 3.0)	71.6	4.06 (1H, t, 3.0)	71.9
2 α	2.19 (1H, dt, 16.0, 3.0)	29.5	2.13 (1H, dt, 16.0, 3.0)	29.5	2.22 (1H, dt, 16.0, 3.0)	29.9
2 β	1.91 (1H, dt, 16.0, 3.0)		1.79 (1H, dt, 16.0, 3.0)		1.89 (1H, dt, 16.0, 3.0)	
3	4.77 (1H, br s)	74.6	4.67 (1H, br s)	74.9	4.83 (1H, t, 2.5)	74.8
4		38.1		38.0		38.5
5	2.96 (1H, dd, 5.0, 3.5)	37.4	3.04 (1H, t, 4.5)	36.6	3.17 (1H, d, 5.0)	35.6
6 α	2.81 (1H, m)	29.7	2.60 (1H, m)	29.5	2.27 (1H, m)	28.9
6 β	2.33 (1H, m)		2.18 (1H, m)		2.85 (1H, m)	
7		174.1		174.0		173.9
8		147.2		143.5		142.3
9		211.4		202.9		206.4
10		54.4		54.9		56.1
11		85.7		91.4	3.31 (1H, d, 2.5)	69.1
12 α	1.48 (1H, d, 14.0)	46.6	1.75 (1H, d, 15.5)	43.8	5.56 (1H, d, 3.0)	76.2
12 β	3.20 (1H, d, 14.0)		3.22 (1H, d, 15.5)			
13		45.2		44.9		49.7
14		86.9		85.9		86.2
15 α	2.81 (2H, m)	34.6	2.73 (2H, m)	34.4	2.81 (1H, d, 18.0)	34.7
15 β					2.85 (1H, d, 18.0)	
16		170.6		169.1		168.2
17	6.31 (1H, s)	79.3	6.28 (1H, s)	79.4	6.53 (1H, d, 3.0)	78.2
18	0.93 (3H, s)	17.4	0.78 (3H, s)	17.5	0.90 (3H, s)	11.3
19	1.04 (3H, s)	19.8	0.95 (3H, s)	19.3	1.04 (3H, s)	20.1
20		121.8		121.2		121.1
21	7.53 (1H, s)	139.7	7.46 (1H, s)	139.6	7.59 (1H, s)	140.4
22	6.41 (1H, br s)	108.4	6.37 (1H, br s)	108.3	6.44 (1H, br s)	108.8
23	7.42 (1H, br s)	143.5	7.34 (1H, br s)	143.8	7.42 (1H, br s)	143.3
28	0.96 (3H, s)	22.8	0.86 (3H, s)	22.5	0.98 (3H, s)	22.8
29	0.88 (3H, s)	27.5	0.77 (3H, s)	27.0	0.86 (3H, s)	27.3
30a	5.53 (1H, br s)	113.7	5.47 (1H, br s)	115.2	5.36 (1H, br s)	114.3
30b	5.25 (1H, br s)		5.23 (1H, br s)		5.19 (1H, br s)	
Ac-3	2.02 (3H, s)	20.7	1.93 (3H, s)	20.5	2.05 (3H, s)	20.8
		168.5		171.0		170.7
Ac-12			2.01 (3H, s)	20.9	1.84 (3H, s)	20.4
				169.9		168.8
OCH ₃	3.64 (3H, s)	51.9	3.60 (3H, s)	51.7	3.67 (3H, s)	51.9
OH-9	4.73(1H, s)					

^a Measured in CDCl₃. ^b Measured in mixture of CDCl₃ and CD₃OD (9:1).

Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40–63 μm , Merck, Darmstadt, Germany). Semipreparative HPLC was performed on a Zorbax SB-C₁₈ (10 μm , Agilent Co., Ltd. Wilmington, DE) column (i.d. 9.4 \times 250 mm), developed with CH₃CN–H₂O (42: 58 \rightarrow 47:53, 30 min) (flow rate, 3.0 mL/min; detection, UV 210 nm) at 30 °C. Precoated silica gel GF254 and HF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC.

Plant Material. The leaves of *Cipadessa cinerascens* (Pell.) Hand.-Mazz. were collected in Xishuangbanna, Yunnan Province, People's Republic of China, in June 2006. The sample was identified by Prof. De-Ding Tao, Kunming Institute of Botany, Chinese Academy of Sciences, and a voucher specimen (KIB 06060081) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and powdered leaves of *C. cinerascens* (14 kg) were extracted three times with 95% EtOH. The extracts were combined and concentrated. The marc was partitioned between H₂O and CHCl₃. The CHCl₃-soluble materials were then subjected to silica gel column chromatography with stepwise petroleum ether–EtOAc (from 1:0 to 1:1) and then petroleum ether–EtOAc–CH₃OH (from 1:1:0 to 1:1:1), giving 10 fractions (A1–A10). White cubic crystals formed in fraction A3, which were then separated by filtration to give cipadassin A (**5**, 2.1 g). Fraction A4 was subjected to passage over a C₁₈ column (MeOH–H₂O, 1:9 \rightarrow 10:0), from which a fraction that eluted with 40% MeOH was purified further by Sephadex LH-20 (CHCl₃–MeOH, 1:1) to afford fraction B1. Fraction B1 was then further purified by preparative TLC and HPLC to give **1** (12 mg), **2** (27 mg), **3** (14 mg), and **4** (20 mg).

Cipatrijugin A (1): white powder; [α]_D²⁵ –14.7 (c 0.43, CHCl₃); IR ν_{max} 2951, 2925, 1737, 1682, 1644, 1380, 1283, 1249, 1196, 1177

cm⁻¹; ¹H and ¹³C NMR data, see Table 1; ESIMS m/z 551 [M + Na]⁺; HRESIMS m/z 551.2248 (calcd for C₂₉H₃₆O₉Na, 551.2257).

Cipatrijugin B (2): white powder; [α]_D²⁵ –17.4 (c 0.97, CHCl₃); IR ν_{max} 3446, 2952, 1737, 1683, 1636, 1436, 1376 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; ESIMS m/z 567 [M + H]⁺; HRESIMS m/z 568.2206 (calcd for C₂₉H₃₆O₁₀Na, 567.2206).

Cipatrijugin C (3): white powder; [α]_D²⁵ +3.6 (c 0.47, CHCl₃); IR ν_{max} 2951, 1743, 1701, 1374, 1285, 1226, 1176 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; ESIMS m/z 609 [M + Na]⁺; HRESIMS m/z 609.2293 (calcd for C₃₁H₃₈O₁₁Na, 609.2311).

Cipatrijugin D (4): white powder; [α]_D²⁵ –82.6 (c 0.66, CHCl₃); IR ν_{max} 2950, 1745, 1688, 1654, 1376, 1244, 1227, 1191 cm⁻¹; ¹H and ¹³C NMR data, see Table 2; ESIMS m/z 609 [M + Na]⁺; HRESIMS m/z 609.2297 (calcd for C₃₁H₃₈O₁₁Na, 609.2311).

Cytotoxicity Testing. Cytotoxicity of compounds **1–5** was determined against A549 (human lung adenocarcinoma) and K562 (human lymphocytic leukemia) cells by a MTT assay.⁹ Doxorubicin (ED₅₀ 59.5 nM) was used as the positive control antitumor drug.

Acknowledgment. The authors thank Prof. D.-D. Tao, Kunming Institute of Botany, Chinese Academy of Sciences, for identification of the plant material.

Supporting Information Available: 1D and 2D NMR spectra for cipatrijugins A–D (1–4) and determination of relative configuration of **1** and **4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (a) Champagne, D. E.; Koul, O.; Isman, M. B.; Scudder, G. G. E.; Towers, G. H. N. *Phytochemistry* **1992**, *31*, 377–394. (b) Akhila,

- A.; Rani, K. In *Progress in the Chemistry of Natural Products*; Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Eds.; Springer: Vienna, 1999; Vol. 78, pp 47–149. (c) Mulholland, D. A.; Coombers, P. H. *Curr. Org. Chem.* **2004**, *4*, 1011–1054. (d) Roy, A.; Saraf, S. *Biol. Pharm. Bull.* **2006**, *29*, 191–201.
- (2) (a) Mitsui, K.; Saito, H.; Yamamura, R.; Fukaya, H.; Hitotsuyanagi, Y.; Takeya, K. *J. Nat. Prod.* **2006**, *69*, 1310–1314. (b) Saewan, N.; Sutherland, J. D.; Chantrapromma, K. *Phytochemistry* **2006**, *67*, 2288–2293. (c) Wang, X.-N.; Yin, S.; Fan, C.-Q.; Wang, F.-D.; Lin, L.-P.; Ding, J.; Yue, J.-M. *Org. Lett.* **2006**, *8*, 3845–3848. (d) Yin, S.; Fan, C.-Q.; Wang, X.-N.; Lin, L.-P.; Ding, J.; Yue, J.-M. *Org. Lett.* **2006**, *8*, 4935–4938. (e) Hay, A.-E.; Ioset, J.-R.; Ahua, K. M.; Diallo, D.; Brun, R.; Hostettmann, K. *J. Nat. Prod.* **2007**, *70*, 9–13. (f) Chen, J. L.; Kernan, M. R.; Jolad, S. D.; Stoddart, C. A.; Bogan, M.; Cooper, R. *J. Nat. Prod.* **2007**, *70*, 312–315.
- (3) Editorial Committee (over 300 members) of the Administration Bureau of Traditional Chinese Medicine. In *Chinese Materia Medica (Zhonghua Bencao)*; Shanghai Science & Technology Press: Shanghai, 1998; Vol. 5, pp 32–33.
- (4) (a) Luo, X.-D.; Wu, S.-H.; Ma, Y.-B.; Wu, D.-G. *Phytochemistry* **2000**, *55*, 867–872. (b) Yuan, X.-H.; Li, B.-G.; Zhou, M.; Qi, H.-Y.; Zhang G.-L. *Org. Lett.* **2005**, *7*, 5051–5053. (c) Ren, Y. -L.; Tang, Q.-R.; Di, Y.-T.; He, H.-P.; Zhang, Z.; Li, S.-L.; Hao, X. J. *Helv. Chim. Acta* **2007**, *90*, 764–768.
- (5) (a) Liang, L.; Zhong, C.-C.; Xiao, Z.-Y. *Zhongcaoyao* **1990**, *21*, 2–4. (b) Liang, L.; Zhong, C.-C.; Xiao, Z.-Y. *Zhongcaoyao* **1991**, *22*, 6–8. (c) Rojatkhar, S. R.; Nagasampagi, B. A. *Phytochemistry* **1994**, *37*, 505–507. (d) Rojatkhar, S. R.; Chiplunkar, Y. G.; Nagasampagi, B. A. *Phytochemistry* **1994**, *37*, 1213–1214. (e) Liang, L.; Zhong, C.-C.; Xiao, Z.-Y. *Zhongcaoyao* **1994**, *25*, 236–237.
- (6) (a) Purushothaman, K. K.; Venkatanarasimhan, M.; Sarada, A. L.; Connolly, J. D.; Rycroft, D. D. *Can. J. Chem.* **1987**, *65*, 35–37. (b) Kehrl, A. R. H.; Taylor, D. A. H.; Miven, M. *Phytochemistry* **1990**, *29*, 153–159. (c) Mulholland, D. A.; Lourine, S. E. *Phytochemistry* **1998**, *47*, 1357–1361. (d) Ismail, I. S.; Ito, H.; Hatano, T.; Taniguchi, S.; Yoshida, T. *Phytochemistry* **2003**, *64*, 1345–1349. (e) Zhang, H.-P.; Wu, S.-H.; Shen, Y.-M.; Ma, Y.-B.; Wu, D.-G.; Qi, S.-H.; Luo, X.-D. *Can. J. Chem.* **2003**, *81*, 253–257. (f) Ismail, I. S.; Ito, H.; Hatano, T.; Taniguchi, S.; Yoshida, T. *Chem. Pharm. Bull.* **2004**, *52*, 1145–1147.
- (7) *Gaussian 03*, revision B.04; Gaussian, Inc.: Pittsburgh, PA, 2003.
- (8) The relative configuration of **4** was also analyzed by molecular models, energy minimized using density functional theory (DFT) at the 3-21G* basis set level in Gaussian 03, which overlaid with key correlations observed in the ROESY NMR spectrum. For details see the Supporting Information.
- (9) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

NP0700624