Lindenane sesquiterpenoid dimers from the roots of Chloranthus fortunei

Lihong Hu
Chinese Academy of Sciences

Lindenane Sesquiterpene Dimers from *Chloranthus fortunei*

Xia-Chang Wang,[†,‡] Yi-Nan Zhang,[†] Li-Li Wang,[†] Shi-Ping Ma,[‡] Jing-Han Liu,[‡] and Li-Hong Hu*,[†]

Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 199 Guo Shou Jing Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China, and College of Traditional Chinese Medicine, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, Jiangsu, People's Republic of China

Received December 30, 2007

Five new lindenane sesquiterpene dimers (**1**−**5**), named shizukaols K−O, and eight known sesquiterpene dimers were isolated from the roots of *Chloranthus fortunei.* The structures of **1**−**5** were elucidated using spectroscopic data, mainly 1D NMR, 2D NMR, and mass spectra.

In the course of searching for biologically active substances from traditional Chinese medicines, lindenane sesquiterpenoids were reported to exhibit antifungal activity[1−3] and moderate cytotoxicity.[4] A series of lindenane dimers have been isolated from *Chloranthus* spp.,[1,5−12] and their complex structures and biological activities have been of interest to natural product chemists. Chloramultilide B, isolated from *Chloranthus spicatus,* was reported to show inhibitory activity against *Candida albicans* and *Candida parapsilosis*.[1] Chlorahololides A and B isolated from *Chloranthus holostegius* exhibited potent and selective inhibition of the delayed rectifier (I_K) K^+ current.[5] Shizukaol B, cycloshizukaol A, and shizukaol F isolated from *Chloranthus japonicus* inhibited the expression of cell adhesion molecules.[13]

Chloranthus fortunei (A. Gray) Solms-Laub (Chloranthaceae) has been used in Chinese folk medicine for the treatment of bone fractures,[14] but the chemical constituents of this plant have not been investigated. Thus, 13 compounds were isolated in our search for structurally unique lindenane dimers from the roots of *C. fortunei.* Five of the compounds were identified as new sesquiterpene dimers (**1**−**5**) and were named shizukaols K−O. The other eight known compounds were 13′-acetylshizukaol C[12] and shizukaols A,[11] B,[10] D,[10] E,[8] F,[8] I,[8] and J.[7] Their structures and relative configurations were elucidated using spectroscopic data, mainly 1D NMR, 2D NMR, and mass spectra.

Results and Discussion

Shizukaol K (**1**) was obtained as a white powder. The molecular formula was determined as $C_{38}H_{44}O_{11}$ from the HRESIMS (*m/z* 699.2784 $[M + Na]^+$), which indicated 17 degrees of unsaturation. The IR spectrum revealed the presence of OH (3469.4 cm^{-1}) and carbonyl (1758.8 cm^{-1}) groups. The ^{13}C NMR and DEPT experiments displayed 38 carbon resonances, which were ascribed to five carbonyl, eight olefinic, one methoxy, six methyl, six methylene, eight methine, and four quaternary carbons (Table 1). The characteristic high-field methylene signals of **1** at δ_H 0.28 (H-2β, m) were diagnostic for the cyclopropane ring of a lindenane sesquiterpene.[7−11] The $^1H-^1H$ COSY spectrum showed two sets of proton spin systems of a 1,2-disubstituted cyclopropane ring (δ_H 0.28, 0.98, 1.85, and 2.05; 0.69, 1.25, 1.50, and 1.55). Therefore, **1** appeared to be a lindenane dimer. Analysis of the ^1H and ^{13}C NMR and mass spectra indicated that **1** was an isomer of 13′-acetylshizukaol C isolated from *Chloranthus japonicus.*[12] Their ^{13}C and ^1H NMR data (Tables 1 and 2) were quite similar except that the olefinic proton of the tiglic acid residue in 13′-acetylshizukaol C (δ_H 6.81, dd) was shifted upfield and sharpened in **1** (δ_H 5.73, br s). Furthermore, the two methyls (δ_H 1.79 and 1.80) of the tiglic acid residue in 13′-acetylshizukaol C were replaced by methyl signals at δ_H 1.94 and 2.19. In the HMBC spectrum of **1** (Figure 1), H-2″ (δ_H 5.73) correlated to C-1″ (δ_C 166.3), C-4″ (δ_C 20.2), and C-5″ (δ_C 27.3). The pair of signals at δ_H 1.94 and 2.19 (Me-4″ and Me-5″) showed correlations to C-1″, C-2″ (δ_C 115.1) and C-3″ (δ_C 158.4), indicating a senecioic acid moiety. A strong cross-peak of C-1″ (δ_C 166.3) with H_2-15′ (δ_H 3.74 and 4.09) determined the senecioic acid residue to be at C-15′. Some key HMBC correlations were observed between H_2-13′ (δ_H 4.80) and C-7′ (δ_C

Table 1. ^{13}C NMR Data (δ) (100 MHz, $CDCl_3$) for Shizukaols K−O (**1**−**5**)

position	1	2	3	4	5
1	25.5, CH	26.3, CH	25.5, CH	25.8, CH	25.6, CH
2	15.7, CH_2	15.8, CH_2	15.7, CH_2	15.8, CH_2	15.7, CH_2
3	24.5, CH	24.7, CH	24.5, CH	24.7, CH	24.6, CH
4	142.1, qC	142.3, qC	141.9, qC	142.3, qC	142.1, qC
5	131.5, qC	131.6, qC	132.0, qC	131.9, qC	131.9, qC
6	40.6, CH	40.5, CH	40.5, CH	40.8, CH	40.6, CH
7	131.1, qC	131.5, qC	131.8, qC	131.4, qC	131.6, qC
8	200.0, qC	200.7, qC	200.3, qC	200.4, qC	200.4, qC
9	80.0, CH	80.3, CH	79.8, CH	79.9, CH	79.8, CH
10	50.9, qC	51.1, qC	50.8, qC	50.9, qC	50.9, qC
11	147.5, qC	147.5, qC	146.3, qC	147.1, qC	146.6, qC
12	170.3, qC	170.9, qC	171.1, qC	171.5, qC	171.6, qC
13	20.2, CH_3	20.2, CH_3	19.8, CH_3	20.3, CH_3	20.1, CH_3
14	15.0, CH_3	14.3, CH_3	15.1, CH_3	15.3, CH_3	15.1, CH_3
15	25.1, CH_2	25.2, CH_2	25.1, CH_2	25.2, CH_2	25.1, CH_2
1′	25.2, CH	25.4, CH	25.2, CH	25.4, CH	25.3, CH
2′	11.8, CH_2	12.1, CH_2	11.7, CH_2	11.7, CH_2	11.7, CH_2
3′	28.1, CH	28.3, CH	28.0, CH	28.0, CH	27.6, CH
4′	76.9, qC	76.9, qC	76.9, qC	76.9, qC	77.0, qC
5′	60.0, CH	60.2, CH	60.4, CH	60.2, CH	60.8, CH
6′	22.4, CH_2	22.0, CH_2	22.2, CH_2	22.3, CH_2	23.0, CH_2
7′	172.0, qC	165.0, qC	165.3, qC	168.5, qC	171.0, qC
8′	93.1, qC	92.5, qC	92.5, qC	93.2, qC	93.3, qC
9′	55.5, CH_2	55.5, CH_2	55.2, CH_2	55.1, CH_2	55.5, CH_2
10′	44.4, qC	44.6, qC	44.6, qC	44.7, qC	44.6, qC
11′	123.4, qC	124.8, qC	124.3, qC	127.2, qC	123.1, qC
12′	171.1, qC	173.5, qC	173.5, qC	172.4, qC	172.3, qC
13′	55.0, CH_2	8.7, CH_2	8.4, CH_2	54.5, CH_2	55.7, CH_2
14′	26.3, CH_3	25.4, CH_3	26.1, CH_3	26.2, CH_3	26.2, CH_3
15′	69.7, CH_2	70.8, CH_2	70.8, CH_2	71.6, CH_2	71.1, CH_2
OMe	52.3, CH_3	52.7, CH_3	52.6, CH_3	52.7, CH_3	52.7, CH_3
1″	166.3, qC	165.5, qC	166.8, qC	171.1, qC	166.9, qC
2″	115.1, CH	144.5, qC	112.5, CH	20.7, CH_3	112.6, CH
3″	158.4, qC	126.9, CH	159.5, qC		159.4, qC
4″	20.2, CH_3	170.4, qC	66.7, CH_2		66.6, CH_2
5″	27.3, CH_3	15.1, CH_3	15.7, CH_3		15.6, CH_3
1‴	170.3, qC				172.4, qC
2‴	20.2, CH_3				28.6, CH_2
3‴					28.6, CH_2
4‴					175.1, qC

* To whom correspondence should be addressed. Tel: +86-21-50272221. Fax: +86-21-50272221. E-mail: simmhulh@mail.shcnc.ac.cn.

† Shanghai Research Center for Modernization of Traditional Chinese Medicine.

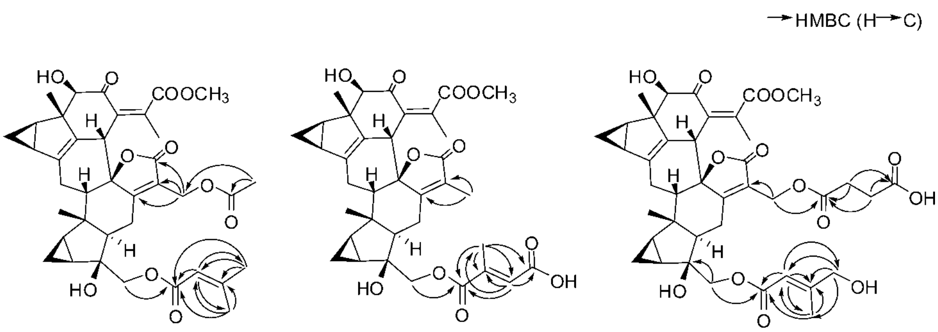
‡ China Pharmaceutical University.

10.1021/np7007544 CCC: $40.75
Published on Web 02/28/2008

Table 2. ^{1}H NMR Data (300 MHz) for Shizukaols K−O (**1**−**5**)

	1	**2**		**3**	**4**	**5**
position	δ_H (J in Hz)[a]	δ_H (J in Hz)[a]	δ_H (J in Hz)[b]	δ_H (J in Hz)[a]	δ_H (J in Hz)[a]	δ_H (J in Hz)[a]
1	2.05 m	2.02 m	2.39 m	2.05 m	2.05 m	1.98 m
2α	0.98 m	0.96 m	1.01 m	1.01 m	0.96 m	1.05 m
2β	0.28 m	0.25 m	0.40 m	0.28 m	0.31 m	0.25 m
3	1.85 m	1.77 m	1.94 m	1.84 m	1.85 m	1.82 m
6	3.91 bs.	3.91 bs.	4.18 bs.	3.88 d	3.91 d	3.86 d
9	3.96 s	3.93 s	4.45 s	3.98 s	3.92 s	3.89 s
13	1.88 s	1.79 s	2.01 s	1.80 s	1.91 s	1.82 s
14	1.01 s	0.99 s	1.34 s	0.99 s	1.00 s	0.97 s
15α	2.75 m	2.72 d	2.95 d	2.75 d (16.5)	2.79 d (14.4)	2.72 m
15β	2.59 m	2.48 m	2.68 m	2.47 d (16.5)	2.55 m	2.39 m
1′	1.55 m	1.55 m	1.54 m	1.59 m	1.60 m	1.53 m
2′α	0.69 m	0.70 m	0.80 m	0.69 m	0.70 m	0.66 m
2′β	1.25 m	1.24 m	1.62 m	1.26 m	1.28 m	1.25 m
3′	1.50 m	1.51 m	1.74 m	1.44 m	1.44 m	1.40 m
5′	1.75 m	1.68 m	2.15 m	1.86 m	1.89 m	1.71 m
6′α	2.32 d (5.7)	2.53 m	2.66 m	2.55 dd	2.32 m	2.53 m
6′β	2.38 d (5.7)	2.58 m	2.85 m	2.61 dd	2.69 m	2.62 m
9′	1.83 m	1.74 m	1.93 m	1.93 m	1.80 m	1.75 m
13′	4.80 dd	1.79 s	1.83 s	1.80 s	4.36 dd	4.87 dd
14′	0.86 s	0.83 s	1.07 s	0.83 s	0.87 s	0.85 s
15′	3.74 m	3.79 d	4.39 d (11.4)	3.78 d (12.0)	3.81 m	3.76 d
	4.09 d (11.7)	4.03 d (11.4)	4.50 d (11.4)	4.21 d (12.0)	4.04 d (11.4)	4.20 d
OMe	3.78 s	3.76 s	3.81 s	3.74 s	3.78 s	3.72 s
2″	5.73 d			6.01 d	2.11 s	6.00 s
3″		6.82 s	7.11 s			
4″	1.94 s			4.18 s		4.17 s
5″	2.19 s	2.28 s	2.52 s	2.12 s		2.10 s
2‴	2.09 s					2.62 m
3‴						2.62 m

[a] Measured in $CDCl_3$. [b] Measured in pyridine-d_5.



1 **2** **5**

Figure 1. Key HMBC correlations of shizukaol K (**1**), shizukaol L (**2**), and shizukaol O (**5**).

172.0), C-11′ (δ_C 123.4), C-12′ (δ_C 171.1) and between H_3-2‴ (δ_C 2.09) and C-1‴ (δ_C 170.3), C-13′ (δ_C 55.0). Thus, **1** has a senecioic acid residue attached to C-15′ rather than tiglic acid as in 13′-acetylshizukaol C.

The relative configuration of **1** was determined by ROESY experiment (Figure 2). ROESY interactions found included H-1/H-9, H-6/H-14, H-6/H-13, H-9′/H-14′, and H-9/H-5′. The ROESY correlations between H-3′ and H_2-15′ indicated that OH-4′ was β-oriented. A ROESY interaction was also observed between H-2″ (δ_H 5.73) and H-4″ (δ_H 1.94) in the senecioic acid substructure. Hence, the structure of **1** was determined to be as shown, and it was named shizukaol K.

Shizukaol L (**2**) was obtained as a white powder, and the molecular formula was determined as $C_{36}H_{40}O_{11}$ from the HRESIMS (m/z 671.2470 $[M + Na]^+$). Compound **2** was clearly recognized as a sesquiterpene dimer from its ^{13}C and ^{1}H NMR data (Tables 1 and 2), which were quite similar to that of **1**. The significant differences observed in the NMR spectra were that the 13′-H_2 signal of **1** was absent and an allyl methyl (δ_H 1.80, 3H) appeared in **2**. The ^{13}C NMR of **2** showed a corresponding methyl signal in the high-field region (δ_C 8.7), characteristic of a 13′-methyl

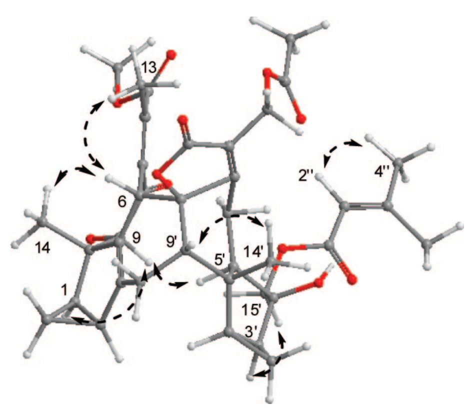
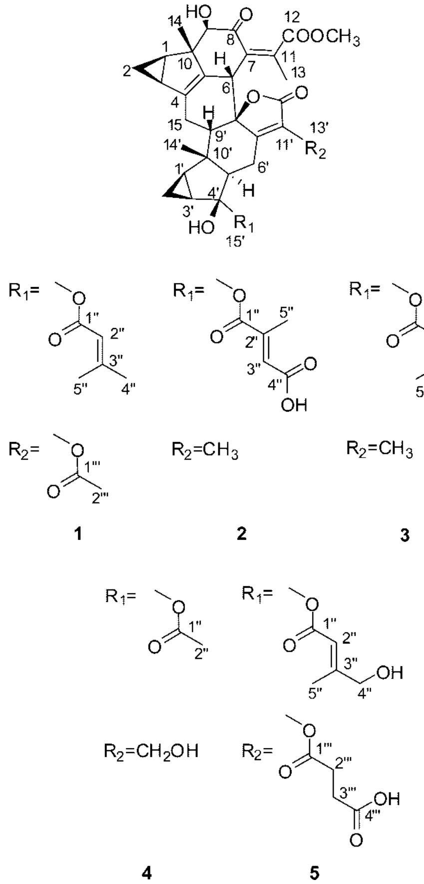


Figure 2. Key ROESY correlations of shizukaol K (**1**).

group in lindenane sesquiterpenes.[8,12–14] Moreover, the senecioic acid moiety of **1** disappeared, and five carbon signals (δ_C 170.4, 165.5, 144. 5, 126.9, 15.1), an olefinic proton (δ_H 6.82, s), and a methyl (δ_H 2.28, s) appeared in the spectra of **2**. In the HMBC



spectrum (Figure 1), H-3″ (δ_H 6.82) correlated with C-1″ (δ_C 165.5), C-2″ (δ_C 144.5), C-4″ (δ_C 170.4), and C-5″ (δ_C 15.1), and H-5″ (δ_H 2.28) correlated with C-1″, C-2″, C-3″ (δ_C 126.9), and C-4″. A strong cross-peak of C-1″ (δ_C 165.5) with H_2-15′ (δ_H 3.79 and 4.03) unambiguously placed the 2-methyl-2-butenedioic acid residue at C-15′. The relative configuration of **2** was also elucidated from correlations observed in the ROESY spectrum. Only the geometry of the double bond on the 2-methyl-2-butenedioic acid moiety could not be established from ROESY correlations, since no ROESY cross-peaks were observed between H-3″ and H-5″. This however suggested that the double bond had *E*-geometry. Pyridine-induced solvent shifts[15] were therefore applied to confirm this result. The significant pyridine-induced solvent shifts (Table 2) for H-5″ ($\Delta\delta$ −0.24) from 4″-OH revealed that they were on the same side; the double bond possessed an *E*-geometry. The structure of **2** was therefore established, and it was named shizukaol L.

Shizukaol M (**3**) was obtained as a yellowish powder, and the molecular formula was determined to be $C_{36}H_{42}O_{10}$ from HRESIMS (*m/z* 657.2672 [M + Na]$^+$). The similarity of the NMR data suggested the same skeleton for **2** and **3** but different substitution at C-15′. There were also signals assigned to five carbons (δ_C 166.8, 112.5, 159.5, 66.7, 15.7), an olefinic proton (δ_H 6.01, s), an oxygen-bearing methylene (δ_H 4.18, s), and a methyl group (δ_H 2.12, s) in the NMR spectra of **3**. Analysis of the key HMBC correlations and comparison of the data with that of Shizukaol I[8] indicated that there was a γ-hydroxysenecioic acid attached to C-15′. Thus, **3** was concluded to be 13′-deoxyshizukaol I, and it was named shizukaol M.

Shizukaol N (**4**) was obtained as a white powder. The molecular formula was determined to be $C_{33}H_{38}O_{10}$ by HRESIMS (617.2366 [M + Na]$^+$). Compound **4** was also identified as a sesquiterpene dimer, with the same skeleton as **1**−**3**, from its ^{13}C and ^{1}H NMR data (Tables 1 and 2). By key HMBC correlations, the signal at δ_H 4.36 (dd, H_2-13′) of **4** was assigned to a hydroxylated methylene at C-13′. The remaining two carbons at δ_C 171.1 (C-1″) and 20.7 (C-2″) and a methyl at δ_H 2.11 (H_3-2″, s) were confirmed to be an acetyl group attached to C-15′ by the HMBC correlations observed between H_3-2″ (δ_H 2.11) and C-1″ (δ_C 171.1) and between H_2-15′ (δ 3.81 and 4.04) and C-1″. Thus, the structure of **4** was established, and it was named shizukaol N.

Shizukaol O (**5**) was obtained as a yellowish powder, and the molecular formula was determined as $C_{40}H_{46}O_{14}$ from the HRESIMS (*m/z* 773.2783 [M + Na]$^+$). The ^{13}C and ^{1}H NMR data (Tables 1 and 2) were quite similar to those of **3**; both had a γ-hydroxysenecioic acid residue on C-15′. The main differences observed were the absence of the characteristic 13′-methyl signal in the high-field region (δ_C 8.4) and the addition of four carbons (δ_C 175.1,172.4, 28.6, 28.6) and two methylene (δ_H 2.62, each two protons overlay) signals in the spectra of **5**. A succinic acid moiety was established by the key HMBC correlations (Figure 1): H_2-2‴ and H_2-3‴ (δ_H 2.62, each two protons overlay) both correlated with C-1‴ (δ_C 172.4) and C-4‴ (δ_C 175.1). A strong cross-peak of C-1‴ (δ_C 172.4) with H_2-13′ (δ_H 4.87) accounted for the succinic acid residue on C-13′. It seemed that the coexistence of the OH on γ-hydroxysenecioic acid and the carboxyl on succinic acid was uncommon. However, methylation of **5** with CH_2N_2 yielded a methyl ester that supported our conclusion. Hence the structure of **5** was determined, and it was named shizukaol O.

The eight known dimeric compounds were identified as 13′-acetylshizukaol C[12] and shizukaols A,[11] B,[10] D,[10] E,[8] F,[8] I,[8] and J[7] by comparison with data in the literature.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were obtained on a Shimadzu UV-2450 UV–visible spectrophotometer. IR spectra were measured on a Nicolet FTIR 750 spectrophotometer. ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, DEPT, HSQC, HMBC, and ROESY spectra were recorded at 300 MHz for ^{1}H, at 100 MHz for ^{13}C, and at 600 MHz for ROESY with Bruker AMX-300/400/600 instruments in $CDCl_3$ or pyridine-d_5 solution. HRESIMS was carried out using Micromass Q-Tof Global mass spectrometers. ESIMS were recorded on a Bruker Esquire 3000 Plus spectrometer. HPLC was performed with a Waters 2695 separation module equipped with a Waters 2996 photodiode array detector and a Kromacil C18 column (4.6 × 150 mm, 0.5 μm). All solvents used were of chemical grade and purchased from the Shanghai Chemical Plant, Shanghai, China. Sephadex LH-20 (25–100 μm) was purchased from Pharmacia. MCI gel CHP 20P (75–150 μm) was purchased from Mitsubishi Chemical Ind., Tokyo, Japan. RP-18 (20–45 μm) was purchased from Fuji Silysia Chemical Ltd. Silica gel (200–300 mesh) for column chromatography was purchased from Qingdao Marine Chemical Ltd., Qingdao, China. Silica gel plates (GF-254) for TLC were purchased from Yantai Huiyou Inc., Yantai, China.

Plant Material. The roots of *C. fortunei* were collected from Zhangzhu Town, Yixing City, Jiangsu Province, China, in May 2007 and identified by Prof. Gui-Xin Chou (Shanghai R&D Center for Standardization of Chinese Medicines). A voucher sample (20070530) was deposited at the Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Shanghai, China.

Extraction and Isolation Procedure. Dried and powdered roots of *C. fortunei* (5 kg) were extracted with MeOH (3 × 30 L) at room temperature. The extract was concentrated under reduced pressure to obtain a dark crude extract (470 g), which was suspended in H_2O, then partitioned with EtOAc to afford the EtOAc solubles (205 g). The EtOAc solubles were then subjected to a column of MCI gel eluted with 30%, 75%, and 90% aqueous MeOH, and 72 g of the 75% aqueous MeOH fraction (a major fraction containing the sesquiterpene dimers) was separated on a silica gel column eluted with petroleum ether−acetone (10:1−1:1) to yield eight fractions, I−VIII. Fraction III (1.2 g) was chromatographed on an RP-18 column, using 75% aqueous MeOH, to yield shizukaol A (31 mg). Fraction IV (11.4g) was first subjected to a silica gel column using $CHCl_3$−acetone (10:1) as eluent, then separated further on Sephadex LH-20 ($CHCl_3$−MeOH (1:1)) and RP-18 (60% aqueous MeOH) columns to yield 13′-acetylshizukaol C (3.6 g), shizukaol E (12 mg), and shizukaol J (6 mg). Fraction V (1.9 g) was subjected to a RP-18 column (65% aqueous MeOH) to yield compound **1** (30 mg). Fraction VI (32 g) was first subjected to a silica gel column using $CHCl_3$−acetone (8:2) as eluent, then purified on Sephadex LH-20 ($CHCl_3$−MeOH (1:1)) and RP-18 (55% aqueous MeOH) columns to yield shizukaol F (10.3 g), shizukaol B (285 mg), shizukaol D (406 mg), compound **3** (460 mg), and compound **4** (32 mg). Fraction VII (2.2 g) was first subjected to a Sephadex LH-20 column ($CHCl_3$−MeOH (1:1)), then further separated on a RP-18 column (55% aqueous MeOH) to yield shizukaol I (120 mg) and compound **2** (11 mg). Fraction VIII (1.7 g) was subjected to a Sephadex LH-20 column ($CHCl_3$−MeOH (1:1)), then separated further on a RP-18 column (50% aqueous MeOH) to yield compound **5** (320 mg).

Shizukaol K (1): white powder; $[\alpha]^{20}_{D}$ −150 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.54); IR (KBr) ν_{max} 3469.4, 2935.2, 1758.8, 1695.1, 1648.9, 1438.7, 1376.9, 1228.5, 1141.7, 1085.7, 1031.7, 993.2 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 699.4 $[M + Na]^+$; HRESIMS *m/z* 699.2784 (calcd for $C_{38}H_{44}O_{11}Na$, 699.2781).

Shizukaol L (2): white powder; $[\alpha]^{20}_{D}$ −118.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.37); IR (KBr) ν_{max} 3446.2, 2950.6, 1731.8, 1646.9, 1436.7, 1376.9, 1278.6, 1195.7, 1124.3, 1085.7, 995.1 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 671.2 $[M + Na]^+$, 647.5 $[M - H]^+$; HRESIMS *m/z* 671.2470 (calcd for $C_{36}H_{40}O_{11}Na$, 671.2468).

Shizukaol M (3): yellowish powder; $[\alpha]^{20}_{D}$ −173.1 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.65); IR (KBr) ν_{max} 3453.9, 2948.7, 1735.6, 1604.5, 1436.7, 1280.5, 1224.6, 1139.7, 1085.7, 995.1 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 1291.3 $[2M + Na]^+$, 633.4 $[M - H]^+$; HRESIMS *m/z* 657.2672 (calcd for $C_{36}H_{42}O_{10}Na$, 657.2676).

Shizukaol N (4): white powder; $[\alpha]^{20}_{D}$ −128.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.20); IR (KBr) ν_{max} 3434.7, 2933.2, 2250.6, 1735.6, 1602.6, 1436.7, 1378.9, 1263.2, 1085.7, 991.2 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 617.2 $[M + Na]^+$, 593.2 $[M - H]^+$; HRESIMS *m/z* 617.2366 (calcd for $C_{33}H_{38}O_{10}Na$, 617.2363).

Shizukaol O (5): yellowish powder; $[\alpha]^{20}_{D}$ −116.8 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.67); IR (KBr) ν_{max} 3448.2, 2935.2, 1731.8, 1604.5, 1436.7, 1280.5, 1224.6, 1157.1, 1085.7, 991.2 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; ESIMS *m/z* 773.1 $[M + Na]^+$, 749.5 $[M - H]^+$; HRESIMS *m/z* 773.2783 (calcd for $C_{40}H_{46}O_{14}Na$, 773.2785).

Methylation of 5. Freshly prepared CH_2N_2 in Et_2O was added to an ethereal solution of **5** (20 mg) at 0 °C, and the reaction mixture was stirred overnight, allowing the temperature to rise to RT. The solvent was evaporated to obtain the C-4‴ methyl ester of **5** (15 mg): yellowish powder; ^{1}H NMR (300Mz, $CDCl_3$) δ_H 5.96 (H-2″, s), 4.77 (H-13′, dd), 4.16 (H-4″, s), 3.97 (H-9, s), 3.91 (H-6, d), 3.72 (CH_3O-12, s), 3.65 (CH_3O-4‴, s), 2.11 (H_3-5″, s), 1.87 (H_3-13, s), 0.98 (H_3-14, s), 0.85 (H_3-14′, s), 0.71 (H-2′α, m), 0.28 (H-2β, m); ESIMS *m/z* 787.3 $[M + Na]^+$, 763.6 $[M - H]^+$.

Acknowledgment. Financial support by the Chinese National Natural Science Foundation of China (NNSF; No. 30371679, 30623008), Chinese National High-tech R&D Programs (2006AA020602, 2006AA02Z156, 2007AA02Z147), and Shanghai Science and Technology Commission (06DZ22028) is gratefully acknowledged.

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, HMBC, ROESY, and ESIMS spectra of shizukaols K−O (**1**−**5**) and the methyl ester of **5** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Xu, Y. -J.; Tang, C. -P.; Ke, C. -Q.; Zhang, J. -B.; Weiss, H. -C.; Gesing, E. -R.; Ye, Y. *J. Nat. Prod.* **2007**, *70*, 1987–1990.
(2) Kawabata, J.; Tahara, S.; Mizutani, J. *Agric. Biol. Chem.* **1981**, *45*, 1447–1453.
(3) Tahara, S.; Fukushi, Y.; Kawabata, J.; Mizutani, J. *Agric. Biol. Chem.* **1981**, *45*, 1511–1512.
(4) Masaaki, U.; Yutaka, K.; Genjiro, K.; Yoshikazu, K.; Shigeo, N.; Chizuko, K.; Tsunematsu, T. *Chem. Pharm. Bull.* **1980**, *28*, 92–102.
(5) Yang, S.-P.; Gao, Z.-B.; Wang, F.-D.; Liao, S.-G.; Chen, H.-D.; Zhang, C.-R.; Hu, G.-Y.; Yue, J.-M. *Org. Lett.* **2007**, *9*, 903–906.
(6) Yang, S.-P.; Yue, J.-M. *Tetrahedron Lett.* **2006**, *47*, 1129–1132.
(7) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1998**, *47*, 231–235.
(8) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1995**, *39*, 121–125.
(9) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1993**, *32*, 1347–1349.
(10) Kawabata, J.; Mizutani, J. *Phytochemistry* **1992**, *31*, 1293–1296.
(11) Kawabata, J.; Fukushi, Y.; Tahara, S.; Mizutani, J. *Phytochemistry* **1990**, *29*, 2332–2334.
(12) Kim, S. U.; Lee, Y. M.; Park, K. D.; Kang, T. H.; Kim, S. E.; Rho, M.-C.; Moon, J. S.; Choi, W. S. U.S. Pat. Appl. Publ. 2007154574 A1, 2007.
(13) Kwon, O. E.; Lee, H. S.; Lee, S. W.; Bae, K.; Kim, K.; Hayashi, M.; Rho, M.-C.; Kim, Y.-K. *J. Ethnopharm.* **2006**, *104*, 270–277.
(14) Editorial Committee of the Administration Bureau of Traditional Chinese Medicine. In *Chinese Materia Medica (Zhonghua Benchao)*; Shanghai Science & Technology: Shanghai, 1998; Vol. 3, pp 449–450..
(15) Demacro, P. V.; Farkas, E.; Doddrell, D.; Mylari, B. L.; Wenkert, E. *J. Am. Chem. Soc.* **1968**, *90*, 5480–5486.

NP7007544