Antimicrobial Diterpenes from *Trigonostemon chinensis*

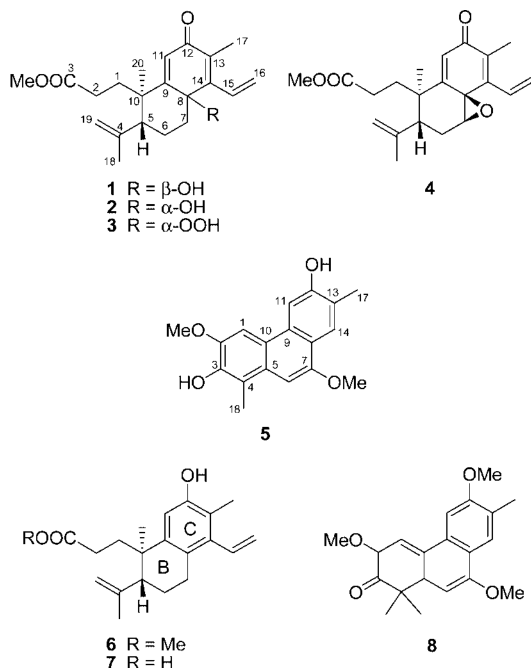
Sheng Yin, Zu-Shang Su, Zhi-Wang Zhou, Lei Dong, and Jian-Min Yue*

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China

Received April 25, 2008

Five new diterpenes, trigonochinenes A−E (**1**−**5**), and two known ones, 3,4-*seco*-sonderianol (**6**) and 3,4-*seco*-sonderianic acid (**7**), were isolated from the aerial part of *Trigonostemon chinensis*. Compounds **1**−**4** possess a rare 3,4-*seco*-cleistanthanic skeleton, and compound **5** is a highly aromatized tetranorditerpene. Structures of these compounds were elucidated by spectroscopic analysis. The antimicrobial activities of compounds **1**−**7** were evaluated against a panel of bacteria and fungi.

The genus *Trigonostemon* (Euphorbiaceae) comprising ca. 50 species is found mainly in India, Malaysia, and middle Asia.[1] Previous investigation on this genus was focused only on *Trigonostemon reidioides* Craib collected from Thailand, which led to the isolation of a phenanthrenone (trigonostemone),[2] modified daphnane (rediocides A−G),[3,4] and a flavonoidal indole alkaloid (lotthanongine).[5] *Trigonostemon chinensis* Merr. form. fungi (Merr.) Y. T. Chang, an evergreen shrub growing only on Hainan Island of China, has not been chemically investigated previously. In the current study, four new 3,4-*seco*-cleistanthanic diterpenes (**1**−**4**) and a new aromatic tetranorditerpene (**5**), together with two known compounds, 3,4-*seco*-sonderianol (**6**) and 3,4-*seco*-sonderianic acid (**7**), were isolated from the aerial part of *T. chinensis*. The antimicrobial activities of compounds **1**−**7** were evaluated against a panel of bacteria and fungi, and some showed remarkable activities against several bacteria. Herein, we report the isolation and structural elucidation of these compounds, as well as their antimicrobial properties.



Results and Discussion

Compound **1** was obtained as a colorless oil with specific rotation of $[\alpha]^{20}_{D}$ +44.0 (*c* 0.07, MeOH). A molecular formula of $C_{21}H_{28}O_4$

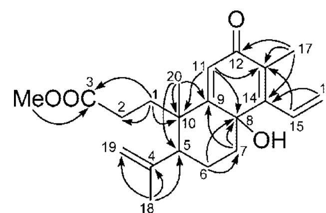


Figure 1. Selected HMBC (→) correlations of **1**.

was assigned for **1** on the basis of HREIMS at *m/z* 344.1970 $[M]^+$ (calcd 344.1988). The IR spectrum displayed absorption bands at 3427, 1736, and 1716 cm^{-1} indicating the presence of OH and carbonyl groups. The ^1H NMR spectrum showed three methyl singlets [δ_H 1.95 (H_3-17), 1.71 (H_3-18), and 1.06 (H_3-20)], a methoxy [δ_H 3.49 (3-OMe)], an AMX terminal vinyl system [δ_H 6.63 (1H, dd, J = 17.6, 12.5 Hz, H-15), 5.65 (1H, dd, J = 12.5, 1.8 Hz, H-16a), and 5.63 (1H, dd, J = 17.6, 1.8 Hz, H-16b)], and an ABX_3 system of an isopropenyl group [δ_H 4.87 (1H, s, H-19a), 4.63 (1H, s, H-19b), and 1.71 (H_3-18)]. The ^{13}C NMR spectrum, in combination with DEPT experiments, resolved 21 carbon resonances attributable to one conjugated carbonyl, one ester carbonyl, four sp^2 quaternary carbons, two sp^2 methines, two sp^2 methylenes, two quaternary sp^3 carbons (one oxygenated), one sp^3 methine, four sp^3 methylenes, and four methyls (one OCH_3). The data indicated that **1** had the same backbone as a coexisting major diterpene, 3,4-*seco*-sonderianol (**6**),[6] which is the only natural diterpene of this type reported. The structural differences between two compounds were the phenol ring C in **6** being oxygenated to a 4-hydroxycyclohexa-2,5-dienone ring in **1**, as the carbon resonances of a highly conjugated ketone carbonyl at δ_C 186.4 and an oxygenated quaternary carbon at δ_C 71.3 were observed in the ^{13}C NMR spectrum of **1**. The HMBC correlations from H_3-17 to C-12, C-13, and C-14 proved the presence of a 12-oxo group, and the HMBC correlations from H_2-6, H_2-7, and H-11 to the oxygenated quaternary carbon placed the OH group at C-8 (Figure 1).

The relative configuration of **1** was determined by a ROESY experiment (Figure 2) and pyridine-induced solvent shifts. The ROESY correlation of H-6β/H-1a indicated that H-6β and the methyl propionate group occupied the 1,3-diaxial bonds of the six-membered ring B and were randomly designated as having a β-configuration, suggesting that the ring B took a chair conformation. The ROESY correlations from H-5 to H-6α, H-6β, and H_3-20 revealed that H-5 was β-oriented. As no convincing evidence was observed in the ROESY spectrum to assign the configuration of 8-OH, the ^1H NMR spectrum of **1** was recorded in C_5D_5N to obtain the pyridine-induced solvent shifts. The solvent shift of H_a-1 ($\Delta\delta_{CDCl_3}-C_5D_5N = \delta_H\ 2.73 - \delta_H\ 3.27 = -0.54$ ppm) indicated that the 8-OH was β-directed.[7] Thus, the structure of **1** was assigned

* Corresponding author. Tel: 86-21-50806718. Fax: 86-21-50806718. E-mail: jmyue@mail.shcnc.ac.cn.

10.1021/np800256x CCC: $40.75 © 2008 American Chemical Society and American Society of Pharmacognosy
Published on Web 07/09/2008

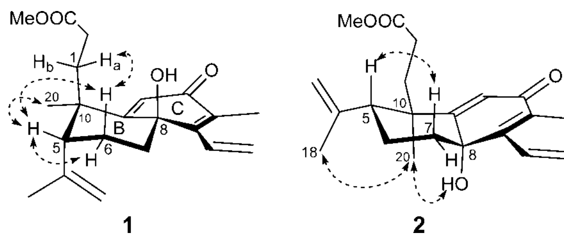


Figure 2. Key ROESY (H↔H) correlations of **1** and **2**.

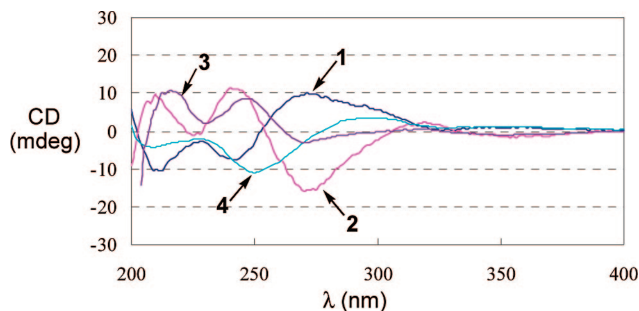


Figure 3. CD curves of compounds **1**−**4**.

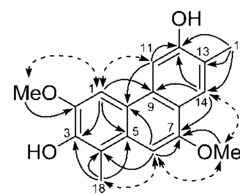


Figure 4. Selected HMBC (↔) and key ROESY (dashed arrows) correlations of **5**.

as methyl 8β-hydroxy-12-oxo-3,4-secocleistanth-9(11),13,15,19(4)-tetraen-3-oate, and it was named trigonochinene A.

Compound **2** was obtained as a pale oil with the specific rotation of $[\alpha]^{20}_{D}$ −22.0 (*c* 0.11, MeOH); $C_{21}H_{28}O_4$ HREIMS. The UV, IR, and ^{1}H NMR data of **2** showed a strong similarity to those of **1**. However, resonances of the C-5 methine and C-20 methyl groups, which were implied by the molecular formula and ^{1}H NMR data, were not observed in the ^{13}C NMR spectrum of **2**. Fortunately, the cross-peaks observed in the HMQC and HMBC experiments assisted in locating the two undetected carbon resonances and allowed the determination of the planar structure of **2**. The relative configuration of **2** was established by a ROESY spectrum (Figure 2), in which the correlations of H-7β/H-5, H_3-20/8-OH, and H_3-20/H_3-18 indicated that **2** was the 8-epimer of **1**. This assignment was supported by their opposite CD curves (Figure 3), which seemed exclusively associated with the C-8 chiral center. Thus, the structure of trigonochinene B (**2**) was assigned as methyl 8α-hydroxy-12-oxo-3,4-secocleistanth-9(11),13,15,19(4)-tetraen-3-oate.

Compound **3** displayed a pseudomolecular ion at *m/z* 361.2012 $[M + H]^+$ consistent with the molecular formula $C_{21}H_{28}O_5$. The ^{13}C NMR data of **3** were similar to those of **1** and **2** except for the severely downfield-shifted resonance of C-8 at δ_C 82.9, indicating that **3** possessed a hydroperoxy group at C-8. This was supported by the proton resonance at δ_H 7.84 (1H, brs) in the ^{1}H NMR assignable to the −OOH, which was devoid of correlations in the HSQC spectrum.[7c] The HMBC correlations from H-6, H-7, H-11, and H-15 to C-8 further supported this conclusion. The configuration of **3** was determined by a ROESY experiment, in combination with its CD spectrum. Particularly, the −OOH was assigned as α-oriented, as the CD curve of **3** bore a resemblance to that of **2**. Compound **3**, when dissolved in solvents such as chloroform, was partially converted into **6** in several days. The structure of **3** was therefore assigned as methyl 8α-hydroperxy-12-oxo-3,4-seco-cleistanth-9(11),13,15,19(4)-tetraen-3-oate, and it was named trigonochinene C.

Compound **4** displayed a molecular ion peak at *m/z* 342.1833 $[M]^+$ consistent with a molecular formula of $C_{21}H_{26}O_4$, two mass units less than that of **1**. The NMR data of **4** resembled those of **1** except that the C-8 at δ_C 57.0 was shifted upfield, and the C-7 at δ_C 63.2 became an oxygenated methine, suggesting that an epoxy ring was formed between C-7 and C-8 in **4**. The HMBC correlations of H-6/C-7 and C-8, H-11/C-8, and H-5/C-7 confirmed this conclusion. The coupling constant of H-7 (dd, J = 3.4, 1.8 Hz) indicated that H-7 was equatorial, and the dihedral angle between H-7 and one of the two H_2-6 protons was close to 90° according to the Karplus equation,[8] suggesting that the epoxy group was β-directed. In a Chem 3D molecular modeling study, the energy-minimized conformation of both the α- and β-epoxy isomers of **4** were calculated for their dihedral angles, and compound **4**, with the β-epoxy, displayed a dihedral angle of ca. 84° between H-7 and H-6a (see the Supporting Information), supporting the above conclusion. This also was supported by the similar CD curves of **4** and **1**. Thus, the structure of **4** was assigned as methyl 7β,8β-epoxy-12-oxo-3,4-secocleistanth-9(11),13,15,19(4)-tetraen-3-oate, and it was named trigonochinene D.

Compound **5** was assigned the molecular formula $C_{18}H_{18}O_4$ (HREIMS) with 10 degrees of unsaturation. The IR bands at 3477, 1600, 1500, and 1483 cm^{-1} indicated the presence of OH and aromatic groups. The ^{1}H NMR spectrum of **5** indicated the presence of four aromatic protons [δ_H 7.61 (s, H-1), 6.78 (s, H-6), 7.73 (s, H-11), and 7.97 (s, H-14)], two methoxy groups [δ_H 3.96 (3H, s) and 3.99 (3H, s)], and two aromatic methyls [δ_H 2.38 (3H, s) and 2.47 (3H, s)]. The ^{13}C NMR and DEPT spectra showed 18 resonances comprising 10 sp^2 quaternary carbons (four oxygenated), four sp^2 methines, two methoxyls, and two methyls. The double bonds accounted for seven out of the 10 degrees of unsaturation; the remaining three double-bond equivalents required **5** to be tricyclic. The ^{1}H NMR spectrum contained only 16 proton resonances, and the two remaining protons in the molecule were attributed to the exchangeable protons of two OH groups. The aforementioned data resembled the spectra of trigonostemonone (**8**),[2] a phenanthrenone previously isolated from this genus. The major structural differences were due to the absence of a 19-methyl group and the A-ring aromatized in **5**. The structure of **5** was further demonstrated by HMBC and ROESY spectra (Figure 4). Trigonochinene E (**5**) represents the highest aromatized tetra-norditerpene reported hitherto from the biosynthetic view.[9,10]

Two known diterpenes, 3,4-*seco*-sonderianol (**6**)[6] and 3,4-*seco*-sonderianic acid (**7**)[6] were identified by comparison of their spectroscopic data with those reported. Compound **7** was isolated as a natural product for the first time.

Compounds **1**−**7** were tested for antimicrobial activity against 11 microorganisms *in vitro*, and the results are summarized in Table 3. All compounds tested were active against *Helicobacter pylori*-SS1 with MICs of 12.5−25 μg/mL, while compounds **1**−**4** and **6** also showed significant inhibitory activities against the drug (metronidazole)-resistant strain of *H. pylori*-ATCC 43504 with MICs of ca. 50 μg/mL. Compounds **3**, **5**, and **6** exhibited selective activities against Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and *Micrococcus luteus* ATCC 9341. In addition, compound **5** showed significant activities against the fungi *Candida albicans* ACTT 1600 and *Microsporum gypseum* with MICs of 12.5 and 6.25 μg/mL, respectively, and compound **6** showed significant inhibitory activity against the Gram-positive bacteria *Bacillus subtilis* CMCC 63501 with an MIC of 3.12 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were recorded

Table 1. ^{1}H NMR Spectroscopic Data for Trigonochinenes A−E (**1**−**5**)[a]

position	1[b]	2[b]	3[b]	4[b]	5[c]
1a	2.73 (m)	2.04 (m)	2.04 (m)	2.43 (m)	7.61 (s)
1b	1.78 (ddd, 13.9, 5.8, 5.8)	1.88 (m)	1.95 (m)	1.70 (m)	
2a	2.30−2.32 (m)	2.32 (m)	2.50 (m)	2.18−2.28 (m)	
2b	2.30−2.32(m)	2.42 (m)	2.42 (m)	2.18−2.28 (m)	
5	2.85 (dd, 8.6, 3.5)	2.04 (m)	2.05 (m)	2.63 (dd, 6.2, 6.1)	
6α	1.40 (m)	1.48 (m)	1.45 (m)	1.98 (m)	6.78 (s)
6β	2.01 (m)	2.32 (m)	2.16 (m)	2.45 (m)	
7α	2.15 (m)	2.30 (m)	2.41 (m)	3.80 (dd, 3.2, 1.8)	
7β	1.66 (m)	1.22 (m)	1.44 (m)		
11	6.14 (s)	6.04 (s)	6.26 (s)	6.46 (s)	7.73 (s)
14					7.97 (s)
15	6.63 (dd, 17.6, 12.5)	6.55 (dd, 18.0, 12.0)	6.58 (dd, 17.7, 12.1)	6.21 (dd, 18.0, 11.8)	
16a	5.65 (dd, 12.5, 1.8)	5.64 (dd, 12.0, 2.1)	5.65 (dd, 17.7, 1.7)	5.59 (dd, 11.8, 1.8)	
16b	5.63 (dd, 17.6, 1.8)	5.55 (dd, 18.0, 2.1)	5.66 (dd, 12.1, 1.7)	5.46 (dd, 18.0, 1.8)	
17	1.95 (3H, s)	1.86 (3H, s)	2.01 (3H, s)	2.00 (3H, s)	2.38 (3H, s)
18	1.71 (3H, s)	1.79 (3H, s)	1.80 (3H, s)	1.61 (3H, s)	2.47 (3H, s)
19 a	4.87 (s)	4.93 (s)	4.77 (s)	4.64 (br s)	2-OMe:
19 b	4.63 (s)	4.77 (s)	4.95 (s)	4.81 (s)	3.96 (3H, s)
20	1.06 (3H, s)	1.38 (3H, s)	1.41 (3H, s)	1.08 (3H, s)	7-OMe:
3-OMe	3.49 (3H, s)	3.66 (3H, s)	3.68 (3H, s)	3.63 (3H, s)	3.99 (3H, s)
8-OH	3.85 (brs)	2.59 (brd, 1.8)			
8-OOH			7.84 (brs)		

[a] Recorded at 400 MHz, δ_H in ppm, J in Hz. [b] Recorded in $CDCl_3$. [c] Recorded in $CDCl_3$ + CD_3OD mixture (ca. 1:1).

Table 2. ^{13}C NMR Spectroscopic Data for Trigonochinenes A−E (**1**−**5**)[a]

position	1[b]	2[b]	3[b]	4[b]	5[c]
1	35.1	33.5	33.7	34.6	101.3
2	29.5	29.1	29.0	29.6	145.7
3	176.5	173.9	174.1	173.9	144.1
4	145.8	145.4	145.0	145.3	117.3
5	51.2	53.5[d]	55.0[d]	47.9	128.1
6	22.9	23.3	23.6	26.4	96.5
7	36.0	38.6	37.7	63.2	153.2
8	71.3	71.4	82.9	57.0	124.8
9	164.9	165.4	162.5	159.1	132.0
10	43.9	44.6	44.6	42.0	119.9
11	126.1	123.5	126.5	131.4	106.3
12	186.4	187.0	186.6	186.3	155.5
13	128.4	129.7	133.6	136.2	125.5
14	156.9	156.4	152.4	148.3	124.8
15	132.3	132.2	131.9	130.0	
16	123.5	123.4	123.3	123.6	
17	12.0	12.2	12.4	13.0	17.0
18	23.9	23.3	23.6[d]	22.1	11.7
19	114.0	115.1	115.3	114.9	2-OMe:
20	20.9	23.0[d]	20.1[d]	24.9	56.4
3-OMe	52.0	51.7	51.8	51.6	7-OMe:
					55.8

[a] Recorded in 100 MHz. [b] As in Table 1. [c] As in Table 1. [d] Signals missed in the ^{13}C NMR spectrum were picked up by the cross-peaks in the HSQC spectrum.

on a Hitachi U-2010 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were measured on Varian Mercury plus 400 and Bruker AM-400 instruments. EIMS and HREIMS (70 eV) were obtained using a Finnigan MAT 95 mass spectrometer. ESIMS was measured on a Finnigan LC Q[DECA] instrument, and HRESIMS was performed on a Waters-Micromass Q-TOF Ultima Global electrospray mass spectrometer. Semipreparative HPLC was performed using a Waters 515 pump with a Waters 2487 detector (254 nm) and a YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm). All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.). Silica gel (20−40 μm, Qingdao Haiyang Chemical Company, Ltd.) was used for CC. RP-18 silica gel (150−200 mesh, Merck) and Sephadex LH-20 gel (Amersham Biosciences) were also used for CC.

Plant Material. Stems and leaves of *T. chinensis* Merr. were collected in May 2005 from Hainan Province, P. R. China, and were identified by Prof. Shi-Man Huang, Research Center of Biology, Hainan University, P. R. China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica (accession number: TCa-2005-1Y).

Extraction and Isolation. The powdered plant material (7.0 kg) was percolated (3 × 5 days) with 90% EtOH at rt to give 260 g of crude extract, which was suspended in 1.5 L water and then partitioned with ethyl acetate to give an ethyl-acetate-soluble fraction (100 g). The ethyl-acetate-soluble fraction was subjected to silica gel column chromatography (CC) eluted successively with a petroleum ether/acetone gradient (100:0 to 0:100) to obtain four fractions (1−4). Fraction 1 was subjected to silica gel CC eluted with petroleum ether/chloroform (1:1) to afford three fractions (1a−1c). Fraction 1a was chromatographed over Sephadex LH-20 gel, followed by semipreparative HPLC using CH_3CN/H_2O (70/30; 3 mL/min) as the mobile phase to give **1** (12 mg) and **2** (21 mg). Fraction 1b was purified by silica gel CC eluted with petroleum ether/ethyl acetate (6:1) to yield **6** (700 mg). Fraction 1c was subjected to a column of RP-18 silica gel eluted with 90% MeOH in water to give a mixture of two major compounds, which were separated by semipreparative HPLC using CH_3CN/H_2O (60/40; 3 mL/min) to give **3** (8 mg) and **4** (6 mg). Fraction 3 was subjected to

Table 3. Antimicrobial Activities of Compounds **1**−**7**

	MIC (μg/mL)[a]							positive control[b]		
microorganism	1	2	3	4	5	6	7	A	B	C
H. pylori-SS1	25	12.5	50	25	25	25	25	0.5		
H. pylori-ATCC 43504	50	25	50	50	>50	50	>50	128		
S. aureus	>50	>50	50	>50	6.25	12.5	>50		12.5	
S. epidermidis	>50	>50	25	>50	6.25	6.25	>50		6.25	
M. luteus	>50	>50	12.5	>50	12.5	6.25	>50		6.25	
E. coli	>50	>50	>50	>50	>50	>50	>50		>50	
Sh. flexneri	>50	>50	>50	>50	>50	>50	>50		>50	
P. aeruginosa	>50	>50	>50	>50	>50	>50	>50		>50	
B. subtilis	>50	>50	12.5	>50	>50	3.12	>50		12.5	
C. albicans	>50	>50	>50	>50	12.5	>50	>50			6.25
M. gypseum	>50	>50	>50	>50	6.25	50	>50			12.5

[a] MIC was defined as the lowest concentration that inhibited visible growth; all the tests were conducted in triplicate; the MIC > 50 μg/mL was defined to be inactive. [b] A−C representing metronidazole, magnolol, and pseudolaric acid B, respectively, were applied as the positive controls.

silica gel CC eluted with CH_3Cl/MeOH (20:1) to obtain the major components and then subjected to a column of Sephadex LH-20 gel eluted with ethanol to afford **5** (50 mg) and **7** (1100 mg).

Antimicrobial Tests. Antibacterial tests against *H. pylori* strains (*Hp*-SS1 or -ATCC 43504 strain) were carried out *in vitro* according to the protocols described previously.[11] *H. pylori* (*Hp*-SS1 or ATCC 43504) cells suspended in saline at a density of 10^8 cfu/mL were inoculated on the agar plates and incubated at 37 °C for 96 h under an atmosphere of 5% O_2, 10% CO_2, and 85% N_2. Blank controls and positive controls were incubated under the same conditions. All tests were conducted in triplicate. The MIC was defined as the lowest concentration of test sample at which visible growth was completely inhibited.

The *in vitro* antibacterial tests against *S. aureus*, *S. epidermidis*, *M. luteus*, *E. coli*, *Sh. flexneri*, *P. aeruginosa*, and *B. subtilis* were conducted as described previously.[12] The microbial cells were suspended in Mueller Hinton broth to form a final density of $5 \times 10^{-5} - 10^{-6}$ cfu/mL and incubated at 37 °C for 18 h under aerobic conditions with the respective compounds, which were dissolved in DMSO. The blank controls of microbial culture were incubated with limited DMSO under the same conditions. DMSO was determined not to be toxic at levels used in the experiments.

The *in vitro* antifungal activities against *C. albicans and M. gypseum* were completed as described previously.[13] The fungi were incubated in Sabouraud dextrose broth at 37 °C for 48 h with the respective compounds, and the positive control was dissolved in DMSO. The blank controls of fungal cultures were incubated with limited DMSO under the same conditions.

Trigonochinene A (1): colorless oil; $[\alpha]^{20}_D$ +44.0 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 298 (3.33), 253 (3.71) nm; IR (KBr) ν_{max} 3427, 2929, 1736, 1716, 1653, 1622, 1437, 1375, 1198, 756 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; EIMS *m/z* 344 $[M]^+$ (38), 326 (9), 301 (24), 257 (30), 239 (100), 229 (50), 163 (86), 91 (48); HREIMS *m/z* 344.1970 (calcd for $C_{21}H_{28}O_4$, 344.1988).

Trigonochinene B (2): pale oil; $[\alpha]^{20}_D$ −22 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 295 (3.70), 256 (4.02) nm; IR (KBr) ν_{max} 3398, 2958, 1738, 1647, 1618, 1437, 1373, 1175, 1009, 893 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; EIMS *m/z* 344 $[M]^+$ (61), 308 (48), 301 (44), 257 (92), 239 (42), 229 (91), 163 (100), 91 (26); HREIMS *m/z* 344.1985 (calcd for $C_{21}H_{28}O_4$, 344.1988).

Trigonochinene C (3): pale yellow oil; $[\alpha]^{20}_D$ −13 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 290 (3.49), 251 (3.72); IR (KBr) ν_{max} 3425, 2929, 1738, 1716, 1635, 1437, 1380, 1197, 1103, 897 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; positive ESIMS *m/z* 383.2 $[M + Na]^+$ (22), 743.3 $[2M + Na]^+$ (100); negative ESIMS *m/z* 341.2 $[M - H_2O - H]^-$; HRESIMS *m/z* 361.2012 $[M + H]^+$ (calcd for $C_{21}H_{29}O_5$, 361.2015).

Trigonochinene D (4): yellow oil; $[\alpha]^{20}_D$ −23 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 271 (3.92) nm; IR (KBr) ν_{max} 2951, 2929, 1738, 1647, 1437, 1383, 1173, 897, 756 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; EIMS *m/z* 342 $[M]^+$ (9), 328 (12), 274 (19), 255 (100), 239 (32), 213 (41), 201 (34); HREIMS *m/z* 342.1833 (calcd for $C_{21}H_{26}O_4$, 342.1831).

Trigonochinene E (5): white powder; $[\alpha]^{20}_D$ 0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 362 (2.72), 287(4.61), 264 (4.67), 235 (4.57) nm; IR (KBr) ν_{max} 3477, 2945, 2835, 1630, 1614, 1600, 1500, 1483, 1292, 1146, 1076, 852 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; EIMS *m/z* 298 $[M]^+$ (100), 283 (78), 255 (59), 240 (38), 149 (19); HREIMS *m/z* 298.1204 (calcd for $C_{18}H_{18}O_4$, 298.1205).

Acknowledgment. Financial support of the Key Project of National Natural Science Foundation (Grant No. 30630072; 30721005) and Shanghai Municipal Scientific Foundation (Grant No. 06DZ22028) of P. R. China is gratefully acknowledged. We thank Professor S. M. Huang, Department of Biology, Hainan University, for the collection and identification of the plant material.

Supporting Information Available: IR, MS, and 1D and 2D NMR spectra of **1**−**5** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Chen, S. K.; Chen, B. Y.; Li, H. *Flora of China* (Zhongguo Zhiwu Zhi); Science Press: Beijing, 1997; Vol. 44 (2), p 162.

(2) Kokpol, U.; Thebpatiphat, S.; Boonyaratavej, S.; Chedchuskulcai, V.; Ni, C. Z.; Clardy, J.; Chaichantipyuth, C.; Chittawong, V.; Miles, D. H. *J. Nat. Prod.* **1990**, *53*, 1148–1151.

(3) Jayasuriya, H.; Zink, D. L.; Singh, S. B.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Slayton, L.; Gregory, L.; Zakson-Aiken, M.; Shoop, W.; Singh, S. B. *J. Am. Chem. Soc.* **2000**, *122*, 4998–4999.

(4) (a) Jayasuriya, H.; Zink, D. L.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Gregory, L.; Shoop, W. L.; Singh, S. B. *J. Nat. Prod.* **2004**, *67*, 228–231. (b) Soonthornchareonnon, N.; Sakayarojkul, M.; Isaka, M.; Mahakittikun, V.; Chuakul, W.; Wongsinkongman, P. *Chem. Pharm. Bull.* **2005**, *53*, 241–243. (c) Tempeam, A.; Thasana, N.; Pavaro, C.; Chuakul, W.; Siripong, P.; Ruchirawat, S. *Chem. Pharm. Bull.* **2005**, *53*, 1321–1323.

(5) Kanchanapoom, T.; Kasai, R.; Chumsri, P.; Kraisintud, K.; Yamasakia, K. *Tetrahedron Lett.* **2002**, *43*, 2941–2943.

(6) Craveiro, A. A.; Silveira, E. R. *Phytochemistry* **1982**, *21*, 2571–2574.

(7) (a) Demarco, P. V.; Farkas, E.; Doddrell, D.; Mylari, B. L.; Wenkert, E. *J. Am. Chem. Soc.* **1968**, *90*, 5480–5486. (b) Su, B.-N.; Misico, R.; Park, E. J.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **2002**, *58*, 3453–3466. (c) Zubía, E.; Ortega, M.; Hernández-Guerrero, C. J.; Carballo, J. L. *J. Nat. Prod.* **2008**, *71*, 608–614.

(8) Paranagama, P. A.; Wijeratne, E. M. K.; Burns, A. M.; Marron, M. T.; Gunatilaka, M. K.; Arnold, A. E.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2007**, *70*, 1700–1705.

(9) Lin, F. W.; Damu, A. G.; Wu, T. S. *J. Nat. Prod.* **2006**, *69*, 93–96.

(10) Ovenden, S. P. B.; Yew, A. L. S.; Glover, R. P.; Ng, S.; Rossant, C. J.; Jr, J. C. R.; Soejarto, D. D.; Buss, A. D.; Butler, M. S. *Tetrahedron Lett.* **2001**, *42*, 7695–7697.

(11) Yin, S.; Fan, C. Q.; Dong, L.; Yue, J. M. *Tetrahedron* **2006**, *62*, 2569–2575.

(12) Yin, S.; Fan, C. Q.; Wang, Y.; Dong, L.; Yue, J. M. *Bioorg. Med. Chem.* **2004**, *12*, 4387–4392.

(13) Yang, S. P.; Dong, L.; Wang, Y.; Wu, Y.; Yue, J. M. *Bioorg. Med. Chem.* **2003**, *11*, 4577–4584.

NP800256X