JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Asperolides A–C, Tetranorlabdane Diterpenoids from the Marine Alga-Derived Endophytic Fungus *Aspergillus wentii* EN-48

Hao-Fen Sun,[†,‡] Xiao-Ming Li,[†] Li Meng,[§] Chuan-Ming Cui,[†,‡] Shu-Shan Gao,[†,‡] Chun-Shun Li,[†] Cai-Guo Huang,*[,§] and Bin-Gui Wang*[,†]

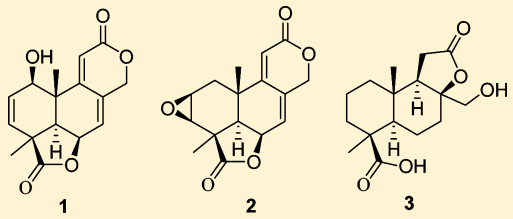
[†]Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Nanhai Road 7, Qingdao 266071, People's Republic of China

[‡]Graduate School of Chinese Academy of Sciences, Yuquan Road 19A, Beijing 100049, People's Republic of China

[§]Department of Biochemistry and Molecular Biology, Second Military Medical University, Xiangyin Road 800, Shanghai 200433, People's Republic of China

S *Supporting Information*

ABSTRACT: Bioassay-guided fractionation of the culture extract of *Aspergillus wentii* EN-48, an endophytic fungus isolated from an unidentified marine brown algal species of the genus *Sargassum*, led to the isolation of three new tetranorlabdane diterpenoids, asperolides A–C (**1**–**3**), and five related derivatives (**4**–**8**). The structures of these compounds were established on the basis of spectroscopic interpretation, and compound **1** was confirmed by X-ray crystallographic analysis. The absolute configuration of **1** was determined by application of the modified Mosher's method. An X-ray structure for wentilactone B (**6**) is also reported. Compounds **1**–**8** were evaluated for cytotoxic and antibacterial activities.



Norditerpenoids have been isolated from various sources including higher plants of the genera *Podocarpus*[1] and *Chamaeceyparis*[2] as well as from fungal species derived from higher plants,[3−6] molded peanuts,[7] and soil samples.[8] These compounds displayed various biological properties including *in vitro* and *in vivo* antifungal,[3,5,6,8] cytotoxic,[8] herbicidal,[9] and plant-growth-regulating activity.[7] As part of our efforts toward the chemical investigation of marine-derived fungi, a variety of structurally interesting and biologically active compounds were isolated and identified from fungal species derived from marine algae,[10−14] mangrove plants,[15] and sediment samples.[16,17] Recently, *Aspergillus wentii* EN-48, an endophytic fungus obtained from an unidentified marine brown algal species of the genus *Sargassum*, attracted our attention. This fungal strain is a common terrestrial species rarely found in the marine environment. The extract of this fungus showed cytotoxicity against several human tumor cell lines in preliminary screening experiments. Bioassay-guided fractionation of the extract from the culture of the fungal strain led to the isolation of three new tetranorditerpenoid derivatives, asperolides A–C (**1**–**3**), together with five related derivatives (**4**–**8**). The known compounds were identified as a tetranorditerpenoid derivative (**4**),[9] wentilactones A (**5**)[7] and B (**6**),[7,9] botryosphaerin B (**7**),[6] and LL-Z1271-β (**8**).[4] It should be noted that the tetranorditerpenoids wentilactones A (**5**) and B (**6**) were previously isolated from a peanut-derived strain of *A. wentii*,[7] and the originally proposed structure for wentilactone B[7] has been revised twice.[9,18] The synthetic work by Barrero and co-workers indicated that the hydroxy group was at C-2 rather than C-3, but a 2α-OH was proposed,[18] while more recent spectroscopic work by Herath and co-workers determined a 2β-OH orientation.[9] Fortunately we obtained a crystal for this compound, and the X-ray crystallographic experiments supported the 2β-OH rather than the 2α-OH in wentilactone B (**6**, Figure 2). The ^{1}H NMR data for H-1 and H-3 were incorrectly assigned in the synthetic work of wentilactone B,[18] and the fully assigned data (recorded in acetone-d_6) for this compound are provided in the Supporting Information (Tables S1 and S2). This paper describes the isolation, structure determination, stereochemical assignment, and cytotoxic activities of these tetranorditerpenoids.

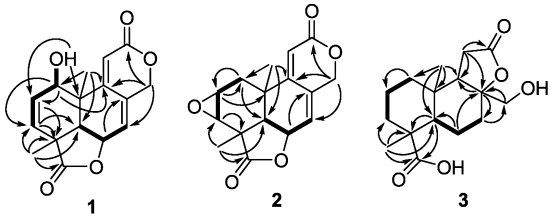
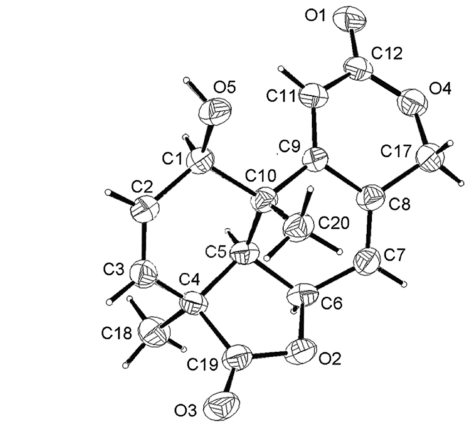


Figure 1. Key HMBC (arrows) and COSY (bold lines) correlations for compounds **1**–**3**.

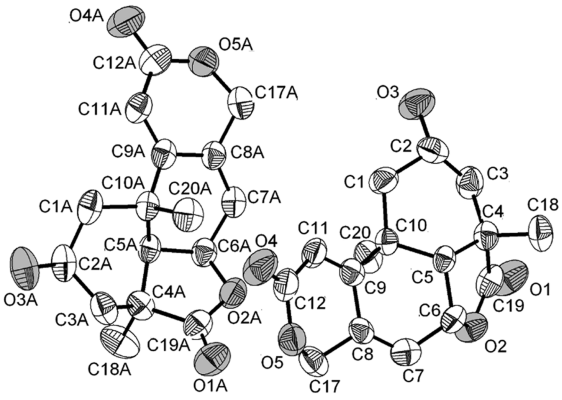
RESULTS AND DISCUSSION

The mycelia and culture broth of *A. wentii* EN-48 were separated by filtration and then exhaustively extracted with acetone and EtOAc, respectively. The combined extracts were

Received: August 14, 2011
Published: January 27, 2012



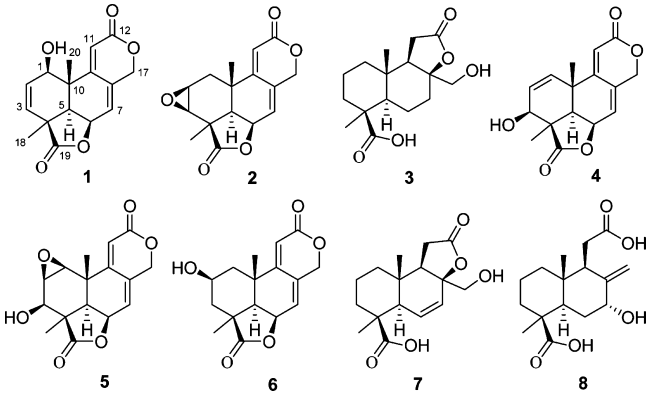
1



6

Figure 2. X-ray crystallographic structures of compounds **1** and **6**.

purified by repeated column chromatography on silica gel, reversed-phase silica gel C_{18}, and Sephadex LH-20, to yield eight tetranorditerpenoids (**1**–**8**).



Compound **1** was obtained as colorless needles. The ESIMS data exhibited pseudomolecular ion peaks at m/z 311 $[M + Na]^+$ and 599 $[2\ M + Na]^+$. The molecular formula was determined as $C_{16}H_{16}O_5$ on the basis of positive HRESIMS data, indicating nine degrees of unsaturation. Detailed inspection of the NMR data (Tables 1 and 2) revealed that

Table 1. ^{1}H NMR Data for Compounds 1–3 (500 MHz, J in Hz)

position	**1**[a]	**2**[b]	**3**[c]
1	4.39, ddd (6.0, 1.6, 1.4)	1.89, d (14.5)	α 1.07, m
		2.34, dd (14.5, 2.2)	β 1.70, m
2	5.62, dd (10.0, 1.1)	3.46, m	1.52, m
			1.86, m
3	5.67, dd (10.0, 2.0)	3.22, d (3.7)	1.08, m
			1.74, m
5	2.36, d (5.0)	1.81, d (4.5)	1.16, dd (10.5, 4.5)
6	5.24, td (4.9, 1.5)	5.00, td (4.6, 1.6)	α 1.87, m
			β 1.46, dd, (14.2, 3.2)
7	6.36, d (4.6)	6.19, m	2.07, dd (14.2, 3.2)
			1.72, m
9			2.24, m
11	6.22, d (1.7)	5.74, d (1.7)	2.90, m
			2.35, d (18.2)
17	4.92, d (13.5)	4.87, d (13.6)	α 3.40, d (10.3)
	5.02, dt (13.5, 1.9)	4.95, dt (13.6, 2.0)	β 3.34, dt (10.3, 2.1)
18	1.29, s	1.53, s	1.24, s
20	0.98, s	1.34, s	0.84, s
1-OH	5.47, d (6.0)		

[a]Recorded in DMSO-d_6. [b]Recorded in $CDCl_3$. [c]Recorded in CD_3OD.

Table 2. ^{13}C NMR Data for Compounds 1–3 (125 MHz)

position	**1**[a]	**2**[b]	**3**[c]
1	68.5, CH	31.0, CH_2	42.0, CH_2
2	134.2, CH	51.4, CH	20.0, CH_2
3	126.6, CH	52.7, CH	38.6, CH_2
4	44.0, C	43.5, C	44.6, C
5	45.9, CH	47.6, CH	53.3, CH
6	69.8, CH	71.6, CH	20.1, CH_2
7	121.8, CH	121.4, CH	31.0, CH_2
8	132.3, C	132.0, C	88.9, C
9	156.0, C	157.9, C	50.8, CH
10	39.5, C	34.6, C	37.5, C
11	113.3, CH	111.4, CH	33.5, CH_2
12	163.4, C	163.2, C	180.3, C
17	68.9, CH_2	69.6, CH_2	69.3, CH_2
18	22.3, CH_3	21.6, CH_3	29.3, CH_3
19	177.6, C	176.5, C	181.0, C
20	15.0, CH_3	24.7, CH_3	14.6, CH_3

[a]Recorded in DMSO-d_6. [b]Recorded in $CDCl_3$. [c]Recorded in CD_3OD.

compound **1** belongs to the tetranorlabdane diterpenoid class and possesses the same carbon skeleton as **4**.[9] The primary differences in the NMR spectroscopic data were that the proton signals for the double bond at C-1/C-2 in **4** moved upfield in **1** (δ_H 6.37 for H-1 and 5.83 for H-2 in **4** versus δ_H 5.62 for H-2 and 5.67 for H-3 in **1**). In addition, the oxygenated carbon signal at δ_C 69.5 (C-3) in **4** slightly shifted upfield to δ_C 68.5 (C-1) in **1**. Furthermore, the methyl proton signal for H_3-20 shifted from δ_H 1.26 in **4** to δ_H 0.98 in **1**, while the corresponding carbon signal shifted from δ_C 28.4 (C-20) in **4** to δ_C 15.0 in **1**. The above evidence suggested that the structure of **1** had a transposed allylic alcohol from C-1 to C-3 compared to **4**. That is, the double bond at C-1/C-2 and the OH group at C-3 in **4** were present at C-2/C-3 and C-1 in **1**, respectively. The observed ^3J HMBC correlations from OH-1 to C-2 and C-10, from H-1 to C-3 and C-9, from H-2 to C-4, from H-3 to C-

dx.doi.org/10.1021/np2006742 | J. Nat. Prod. 2012, 75, 148–152

5, from H_3-18 to C-3, C-5, and C-19, and from H_3-20 to C-1, C-5, and C-9 (Figure 1) allowed the planar structure of **1** to be confirmed.

The relative configuration of **1** was determined by a NOESY experiment. The key NOE correlations from H_3-18 to H-1, H-5, and H-6 in the NOESY spectrum indicated the cofacial orientation relative to H-1, H-5, H-6, and H_3-18, while the correlation from H_3-20 to 1-OH placed these two groups on the opposite face. An X-ray crystallographic experiment (Figure 2) confirmed the structure and relative configuration of **1** as depicted.

The absolute configuration of **1** was determined by the modified Mosher's method and a CD experiment. Treatment of **1** with (*R*)-(−)-α- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPA-Cl) gave the (*S*)- and (*R*)-MTPA esters **1s** and **1r**, respectively.[19] The ^{1}H NMR signals of the two MTPA esters were assigned on the basis of their ^{1}H−^{1}H COSY spectra, and the $\Delta\delta_{H(S-R)}$ values were then calculated (Figure 3). The results indicated that the absolute

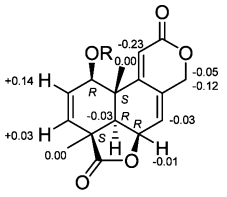


Figure 3. Values of $\Delta\delta_{H(S-R)}$ (measured in acetone-d_6) of the MTPA esters of compound **1**.

configuration of C-1 was *R*. Therefore, the absolute configurations at C-4, C-5, C-6, and C-10 were deduced as *S*, *R*, *R*, and *S*, respectively. Compounds **1** and **6** were also evaluated by CD spectroscopy, and these two compounds displayed negative Cotton effects at 258 and 261 nm, respectively, which are similar to the previously reported minimum for wentilactone B (**6**),[7] indicating that the absolute configuration of compound **1** was 4*S*, 5*R*, 6*R*, and 10*S*, the same as depicted for wentilactone B.[7,9] This assignment is consistent with the configuration as determined by the modified Mosher's method. On the basis of the above evidence, the structure of **1** was determined, and the trivial name asperolide A was assigned to this compound.

Compound **2** was assigned the molecular formula $C_{16}H_{16}O_5$ (nine unsaturations), the same as that of **1**, on the basis of positive HRESIMS data. The NMR spectroscopic data (Tables 1 and 2) differed from those of **1** mainly in the absence of the signals for the oxygenated CH at C-1 and the double bond at C-2/C-3. Instead, resonances corresponding to a CH_2 unit at C-1 (δ_H 1.89 and 2.34 and δ_C 31.0) and an epoxide group at C-2/C-3 (δ_H 3.46/3.22 and δ_C 51.4/52.7) were present. The observed COSY and HMBC correlations (Figure 1) supported the above deduction. The relative configuration of **2** was also determined from the NOESY spectrum. The observed NOE correlations among H-2, H-3, H-5, H-6, and H_3-18 suggested the α-orientation for these protons. Compound **2** was assigned the trivial name asperolide B. Similar to **1** and wentilactone A (**5**),[7] the CD spectrum of **2** exhibited a negative Cotton effect at 260 nm, suggesting the same absolute configuration for the backbone of these compounds. With the 2β,3β-epoxide presented in the structure, the absolute configuration of compound **2** is assigned as 2*S*, 3*R*, 4*R*, 5*R*, 6*R*, and 10*S*.

Compounds **3** and **7** were obtained as a colorless mixture. Attempts to separate the two compounds by different CC steps using various solvent systems failed. HPLC purification of these metabolites was hampered by the lack of a conjugated system, which made these compounds unsuitable for separation using the available UV detector. Alternatively, attempts to perform hydrogenation of the mixed sample with H_2 + Pd/C in THF did not yield the desired pure compound **3**, possibly due to instability of the lactone ring under the hydrogenation conditions. On the basis of their ^{1}H and ^{13}C NMR spectra, the ratio of compounds **3** and **7** was deduced as 3:4 in the mixture. Most of the NMR signals were duplicated and well-separated. Aided by 2D NMR experiments including ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, the structure elucidation and assignments of the chemical shifts for the individual compounds were successfully carried out. The structure of compound **7** was readily determined as botryosphaerin B, a tetranorlabdane diterpenoid recently isolated from the solid-phase culture of the endophytic fungal strain *Botryosphaeria* sp. MHF that was obtained from the plant *Maytenus hookeri*.[6] After that, the structure elucidation of **3** was relatively straightforward due to the close relationship with compound **7**.

Compound **3** was found to have the molecular formula $C_{16}H_{24}O_5$ (five unsaturations), with two hydrogen atoms more than **7** on the basis of negative ion HRESIMS data. A detailed comparison of the ^{1}H and ^{13}C NMR data revealed that the double bond at C-6/C-7 in **7**[6] was absent in **3**. This was evidenced by the fact that the two downfield methine proton signals at δ_H 6.67 and 5.71, derived from the double bond at C-6/C-7 in **7**,[6] were missing in the ^{1}H NMR spectrum of **3**. Instead, proton signals appeared upfield, and two CH_2 groups, at δ_H 1.87 and 1.46 (H_2-6) and at δ_H 2.07 and 1.72 (H_2-7), were observed (Table 1). This observation was strongly supported by the facts that the two sp^2 methine carbon signals at δ_C 135.9 (C-6) and 122.6 (C-7) in **7**[6] were replaced by two sp^3 methylene carbon signals at δ_C 20.1 (C-6) and 31.0 (C-7) in **3** (Table 2). In combination with the analyses of the COSY and HMBC correlations (Figure 1), the structure of compound **3** was established. The NOESY spectrum indicated that H-5, H-9, H_2-17, and H_3-18 adopt an α-orientation. The structure of **3** was named asperolide C.

Compounds **1**−**8** were assayed for their cytotoxic activities against HeLa, HepG2, MCF-7, MDA-MB-231, NCI-H460, SMMC-7721, and SW1990 tumor cell lines, with fluorouracil (5-Fu) and adriamycin (ADM) as positive controls. Although none of these compounds have significant activity ($IC_{50} \leq 10$ μM), compounds **1**, **2**, and **4**−**6** exhibited weak activity against various tumor cell lines, with IC_{50} values lower than 5-Fu, but higher than ADM (Table S3, Supporting Information), with **6** being regarded as the most potent among the tested compounds (IC_{50} = 17 μM).

Compounds **1**, **4**−**6**, and **8** were further evaluated for antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *P. fluorescens*, and *Bacillus subtilis*, as well as antifungal activity against *Candida albicans* using the disk diffusion method. Compound **4** was found to possess considerable antifungal activity against *C. albicans* with an MIC value of 16 μg/mL, while the other four displayed only weak activity against *C. albicans* (data not shown).

dx.doi.org/10.1021/np2006742 | *J. Nat. Prod.* 2012, 75, 148−152

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on an Optical Activity AA-55 polarimeter. UV spectra were recorded on a Lengguang Gold S54 photometer. CD spectra were acquired on a Chirascan spectropolarimeter. IR spectra were determined on a Nicolet NEXUE 470 using KBr discs. 1D and 2D NMR spectra were acquired on a Bruker Avance 500 MHz spectrometer. Mass spectra were determined on a VG Autospec 3000 or an API QSTAR Pulsar 1 mass spectrometer. Commercially available Si gel (200−300 mesh, Qingdao Haiyang Chemical Co.), Lobar LiChroprep RP-18 (40−63 μm, Merck), and Sephadex LH-20 (Pharmacia) were used for open column chromatography.

Fungal Material. The endophytic fungus *Aspergillus wentii* EN-48 was isolated from the fresh tissue of the surface-sterilized marine brown algal species of the genus *Sargassum*. Fungal identification was carried out using a molecular biological protocol by DNA amplification and sequencing of the ITS region as described previously.[20] The sequence data obtained from the fungal strain have been deposited at GenBank with accession no. HM014129. A BLAST search result showed that the sequence was the most similar (99%) to the sequence of *Aspergillus wentii* (compared to EF652157.1 GI: 158535354). The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences.

Fermentation, Extraction, and Isolation. The fresh mycelia of *A. wentii* EN-48 were grown on PDA medium at 28 °C for 6 days and then were inoculated into 1000 mL flasks containing 300 mL of PDB medium (500 mL seawater, 20 g glucose, 5 g peptone, 3 g yeast extract, and potato 20 g/L, distilled water added up to 1000 mL, pH 6.5−7.0) for 30 days at room temperature.

For chemical investigations, mycelia and the culture broth of *A. wentii* EN-48 were separated by filtration and then homogenized using a Waring blender and exhaustively extracted with Me_2CO and EtOAc, respectively. Because the TLC and HPLC profiles of the two extracts were nearly identical, they were combined before further separation. The combined extract was subjected to CC over silica gel eluted with different solvents of increasing polarity from petroleum ether (PE) to MeOH to yield seven fractions (Frs. 1−7) on the basis of TLC analysis. Further purification of Fr. 4 (2.8 g) by CC on silica gel with a PE/EtOAc gradient (from 10:1 to 1:1) and Sephadex LH-20 (MeOH) afforded **1** (15.6 mg), **4** (5.8 mg), and **8** (66.3 mg). Fr. 5 (1.7 g) was further purified by CC on silica gel eluted with a PE/EtOAc gradient (from 10:1 to 1:1), on Sephadex LH-20 (MeOH), and by preparative TLC (plate: 20 × 20 cm, developing solvents: PE/EtOAc, 1:1) to obtain **2** (5.2 mg). Purification of Fr. 6 (1.3 g) on silica gel (PE/EtOAc, 1:1) and Sephadex LH-20 (MeOH) yielded **5** (60.5 mg) and **6** (30.5 mg). A mixture of compounds **3** and **7** (20.5 mg) was obtained from Fr. 7 (2.3 g) by CC on silica gel eluted with a $CHCl_3$−MeOH gradient (from 80:1 to 5:1) and reversed-phase silica gel C_{18} (MeOH/H_2O, 4:1).

*Asperolide A (**1**):* colorless crystals (MeOH); mp 263−264 °C; $[\alpha]_D^{25}$ −488 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.23), 257 (4.25) nm; CD (*c* 0.23, MeOH) λ_{max} ($\Delta\varepsilon$) 258 (−57.4) nm; IR (KBr) ν_{max} 3400, 2983, 2935, 1759, 1713, 1653, 1377, 1369, 1241, 1200, 1162, 1105, 1066, 1025, 985, 942 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 311 $[M + Na]^+$, 599 $[2 M + Na]^+$; HRESIMS *m/z* 289.1079 $[M + H]^+$ (calcd for $C_{16}H_{17}O_5$, 289.1076).

*Asperolide B (**2**):* colorless crystals (MeOH); mp 243−244 °C; $[\alpha]_D^{25}$ −142 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.43), 257 (4.67) nm; CD (*c* 1.31, MeOH) λ_{max} ($\Delta\varepsilon$) 260 (−9.6) nm; IR (KBr) ν_{max} 2982, 2931, 1775, 1726, 1646, 1387, 1258, 1204, 1141, 1081, 1040, 986, 922 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 289 $[M + H]^+$, 311 $[M + Na]^+$, 599 $[2 M + Na]^+$; HRESIMS *m/z* 289.1082 $[M + H]^+$ (calcd for $C_{16}H_{17}O_5$, 289.1076).

*Asperolide C (**3**):* colorless mixture with **7**; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 295.1545 $[M - H]^-$ (calcd for $C_{16}H_{23}O_5$, 295.1546).

*Compound **4**:* colorless needles (MeOH); mp 251−252 °C; $[\alpha]_D^{25}$ −267 (*c* 0.17, MeOH), lit. −262 (*c* 0.57, $CHCl_3/CH_3OH$, 9:1);[9] UV (MeOH) λ_{max} (log ε) 201 (4.43), 257 (4.67) nm; CD (*c* 0.21, MeOH) λ_{max} ($\Delta\varepsilon$) 260 (−50.4) nm; IR (KBr) ν_{max} 2983, 2930, 1775, 1726, 1646, 1387, 1258, 1204, 1141, 1081, 1040, 986, 922 cm^{-1}; ^1H and ^{13}C NMR data, see Tables S1 and S2; ESIMS *m/z* 289 $[M + H]^+$, 311 $[M + Na]^+$, 599 $[2 M + Na]^+$; HRESIMS *m/z* 289.1082 $[M + H]^+$ (calcd for $C_{16}H_{17}O_5^+$, 289.1076).

*Wentilactone A (**5**):* colorless prisms; mp 251−252 °C; $[\alpha]_D^{25}$ −248 (*c* 0.19, MeOH); CD (*c* 0.30, MeOH) λ_{max} ($\Delta\varepsilon$) 260 (−31.2) nm; ESIMS *m/z* 303 $[M - H]^-$; ^1H and ^{13}C NMR data, see Tables S1 and S2.

*Wentilactone B (**6**):* colorless crystals; mp 271−272 °C; $[\alpha]_D^{25}$ −214 (*c* 0.58, MeOH), lit. −236 (*c* 0.58, $CHCl_3/CH_3OH$, 9:1);[9] CD (*c* 0.17, MeOH) λ_{max} ($\Delta\varepsilon$) 261 (−59.6) nm; ESIMS *m/z* 291 $[M + H]^+$; ^1H and ^{13}C NMR data, see Tables S1 and S2.

*LL-Z1271-β (**8**):* colorless prisms; mp 213-215 °C; $[\alpha]_D^{25}$ +24 (*c* 0.97, MeOH), lit. +28 (*c* 0.36, $CHCl_3/CH_3OH$, 9:1);[9] ESIMS *m/z* 295 $[M - H]^-$; ^1H and ^{13}C NMR data, see Tables S1 and S2.

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of Compound 1. To a stirred solution of **1** (2.3 mg) in pyridine (400 μL) was added 4-(dimethylamino)pyridine (2 mg) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl, 10 μL). The mixture was stirred at room temperature for 10 h, and the reaction was stopped by adding 0.2 mL of H_2O. The reaction mixture was then passed through a disposable pipet packed with silica gel and eluted with PE/EtOAc (5:1) to give the respective (*R*)-Mosher ester **1r** (2.0 mg): ^1H NMR (acetone-d_6, 500 MHz) δ 5.61 (1H, dd, *J* = 10.0, 1.2 Hz, H-2), 5.96 (1H, dd, *J* = 10.0, 2.1 Hz, H-3), 2.75 (1H, d, *J* = 5.0 Hz, H-5), 5.36 (1H, td, *J* = 5.0, 1.6 Hz, H-6), 6.44 (1H, d, *J* = 4.6 Hz, H-7), 5.47 (1H, d, *J* = 1.6 Hz, H-11), 5.09 (1H, d, *J* = 13.4 Hz, H_a-17), 4.98 (1H, dt, *J* = 13.4, 1.8 Hz, H_b-17).

Treatment of **1** (2.5 mg) with (*R*)-MTPA-Cl (10 μL) with the same procedure yielded the corresponding (*S*)-Mosher ester **1s** (2.2 mg): ^1H NMR (acetone-d_6, 500 MHz) δ 5.75 (1H, dd, *J* = 10.0, 1.2 Hz, H-2), 5.99 (1H, dd, *J* = 10.0, 2.1 Hz, H-3), 2.72 (1H, d, *J* = 5.0 Hz, H-5), 5.35 (1H, td, *J* = 5.0, 1.6 Hz, H-6), 6.41 (1H, d, *J* = 4.6 Hz, H-7), 5.24 (1H, d, *J* = 1.6 Hz, H-11), 4.97 (1H, d, *J* = 13.4 Hz, H_a-17), 4.93 (1H, dt, *J* = 13.4, 1.8 Hz, H_b-17).

X-ray Crystallographic Analysis of Compounds 1 and 6.[21] All crystallographic data were collected on a Srigaku Mercury CCD/AFCR diffractometer equipped with graphite-monochromatic Mo Kα radiation (λ = 0.71073 Å) at 293(2) K. The data were corrected for absorption by using the program SADABS.[22] The structure was solved by direct methods with the SHELXTL software package.[23] All non-hydrogen atoms were refined anisotropically. The H atoms were located by geometrical calculations, and their positions and thermal parameters were fixed during the structure refinement. The structure was refined by full-matrix least-squares techniques.[24]

*Crystal data for compound **1**:* $C_{16}H_{16}O_5$, fw = 288.29, monoclinic space group *C*2, unit cell dimensions *a* = 17.48(4) Å, *b* = 7.990(2) Å, *c* = 12.93(3) Å, *V* = 1335(5) $Å^3$, α = γ = 90°, β = 132.34(3)°, *Z* = 4, d_{calcd} = 1.434 mg/m^3, crystal dimensions 0.20 × 0.20 × 0.20 mm, μ = 0.107 mm^{-1}, *F*(000) = 608. The 5230 measurements yielded 2868 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0922 and wR_2 = 0.2362 [$I > 2\sigma(I)$].

*Crystal data for compound **6**:* $C_{16}H_{18}O_5$, fw = 290.30, triclinic space group *P*1, unit cell dimensions *a* = 7.7085(1) Å, *b* = 9.851(2) Å, *c* = 10.445(2) Å, *V* = 685.8(2) $Å^3$, α = 80.90(3)°, β = 69.55(3)°, γ = 67.39(3)°, *Z* = 2, d_{calcd} = 1.406 mg/m^3, crystal dimensions 0.20 × 0.20 × 0.20 mm, μ = 0.104 mm^{-1}, *F*(000) = 308. The 5155 measurements yielded 2359 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0731 and wR_2 = 0.2007 [$I > 2\sigma(I)$].

Cytotoxicity Assays. The cytotoxic activities against the HeLa (human epithelial carcinoma), HepG2 (human hepatocellular liver carcinoma), MCF-7 (human breast adenocarcinoma), MDA-MB-231 (human breast cancer), NCI-H460 (human non-small-cell lung cancer), SMMC-7721 (human hepatoma), and SW1990 (human pancreatic cancer) cell lines were determined according to previously reported methods.[25,26] Fluorouracil (5-Fu) and adriamycin were used as positive controls.

dx.doi.org/10.1021/np2006742 | *J. Nat. Prod.* 2012, 75, 148−152

Antimicrobial Assays. The antibacterial activities against the methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *P. fluorescens*, and *Bacillus subtilis*, as well as antifungal activity against *Candida albicans*, were determined (at 100 μg/disk) using the disk diffusion method.[27] The MIC measurements of the isolated components were performed by the broth microdilution method.[28] Briefly, an inoculum level of 5×10^5 cfu/mL and a range of sample concentrations of 1024−1 μg/mL were used. The MIC was determined after the microtiter plates were incubated for 18 h at 35 °C in an ambient air incubator. Ampicillin was used as positive control in the antibacterial test against methicillin-resistant *S. aureus*, *P. fluorescens*, *P. aeruginosa*, and *S. epidermidis* (with MICs 8.0, 2.0, 4.0, and 4.0 μg/mL, respectively), while nystatin was used as positive control in the antifungal assay against *C. albicans* (MIC 2.0 μg/mL).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Selected 1D and 2D NMR spectra of compounds **1**−**3**, the fully assigned ^{1}H and ^{13}C NMR data (Tables S1 and S2) as well as the cytotoxic activity (Table S3) of compounds **1**−**8**, and X-ray crystallographic files of compounds **1** and **6** (in CIF format). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel and Fax: 86-532-82898553. E-mail: huangcaig@hotmail.com (C.-G.H.), wangbg@ms.qdio.ac.cn (B.-G.W.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors sincerely thank Drs. Y.-F. Li, H.-M. Guo, and F.-F. Jian at Weifang University for X-ray crystallographic analysis and Profs. W.-P. Su, Z. Lin, Z.-N. Chen, J. Zhang, and C.-N. Chen as well as Drs. J. Xu, Y.-J. Wang, and C.-B. Ma at Fujian Institute of Research on the Structure of Matter of the Chinese Academy of Sciences for helpful discussion. This work was financially supported by the Ministry of Science and Technology (2011AA09070110 and 2010CB833802) and by the Natural Science Foundation of China (30970293 and 30910103914).

■ REFERENCES

(1) Hembree, J. A.; Chang, C.; McLaughlin, J. L.; Cassady, J. M.; Watts, D. J.; Wenkert, E.; Fonseca, S. F.; de Paiva Campello, J. *Phytochemistry* **1979**, *18*, 1691−1694.
(2) Banerjee, A. K.; Laya, M. S.; Mora, H. R.; Cabrera, E. V. *Curr. Org. Chem.* **2008**, *12*, 1050−1070.
(3) Ellestad, G. A.; Evans, R. H. Jr.; Kunstmann, M. P.; Lancaster, J. E.; Morton, G. O. *J. Am. Chem. Soc.* **1970**, *92*, 5483−5489.
(4) Ellestad, G. A.; Evans, R. H. Jr.; Kunstmann, M. P. *Tetrahedron Lett.* **1971**, *12*, 497−500.
(5) Hosoe, T.; Nozawa, K.; Lumley, T. C.; Currah, R. S.; Fukushima, K.; Takizawa, K.; Miyaji, M.; Kawai, K. *Chem. Pharm. Bull.* **1999**, *47*, 1591−1597.
(6) Yuan, L.; Zhao, P. J.; Ma, J.; Lu, C. H.; Shen, Y. M. *Helv. Chim. Acta* **2009**, *92*, 1118−1125.
(7) Dorner, J. W.; Cole, R. J.; Springer, J. P.; Cox, R. H.; Cutler, H.; Wicklow, D. T. *Phytochemistry* **1980**, *19*, 1157−1161.
(8) Pettit, G. R.; Tan, R.; Herald, D. L.; Hamblin, J.; Pettit, R. K. *J. Nat. Prod.* **2003**, *66*, 276−278.
(9) Herath, H. M. T. B.; Herath, W. H. M. W.; Carvalho, P.; Khan, S. I.; Tekwani, B. L.; Duke, S. O.; Tomaso-Peterson, M.; Nanayakkara, N. P. D *J. Nat. Prod.* **2009**, *72*, 2091−2097. In this reference, the authors described a negative Cotton effect at 295 nm and assigned a 6*S* configuration for wentilactone B, although a 6*R* configuration was illustrated in the structure. However, the previous report[7] and our experimental data showed a negative Cotton effect at 260 and 261 nm, respectively, and suggested a 6*R* configuration for this compound.
(10) Gao, S. S.; Li, X. M.; Li, C. S.; Proksch, P.; Wang, B. G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2894−2897.
(11) Gao, S. S.; Li, X. M.; Du, F. Y.; Li, C. S.; Proksch, P.; Wang, B. G. *Mar. Drugs* **2011**, *9*, 59−70.
(12) Cui, C. M.; Li, X. M.; Meng, L.; Li, C. S.; Huang, C. G.; Wang, B. G. *J. Nat. Prod.* **2010**, *73*, 1780−1784.
(13) Cui, C. M.; Li, X. M.; Li, C. S.; Proksch, P.; Wang, B. G. *J. Nat. Prod.* **2010**, *73*, 729−733.
(14) Cui, C. M.; Li, X. M.; Li, C. S.; Sun, H. F.; Gao, S. S.; Wang, B. G. *Helv. Chim. Acta* **2009**, *92*, 1366−1370.
(15) Liu, D.; Li, X. M.; Meng, L.; Li, C. S.; Gao, S. S.; Shang, Z.; Proksch, P.; Huang, C. G.; Wang, B. G. *J. Nat. Prod.* **2011**, *74*, 1787−1791.
(16) Gao, S. S.; Li, X. M.; Zhang, Y.; Li, C. S.; Cui, C. M.; Wang, B. G. *J. Nat. Prod.* **2011**, *74*, 256−261.
(17) Li, C. S.; An, C. Y.; Li, X. M.; Gao, S. S.; Cui, C. M.; Sun, H. F.; Wang, B. G. *J. Nat. Prod.* **2011**, *74*, 1331−1334.
(18) Barrero, A. F.; Herrador, M. M.; del Moral, J. F. Q.; Valdivia, M. V. *Org. Lett.* **2002**, *4*, 1379−1382.
(19) Wang, B. G.; Ebel, R.; Wang, C. Y.; Wray, V.; Proksch, P. *Tetrahedron Lett.* **2002**, *43*, 5783−5787.
(20) Wang, S.; Li, X. M.; Teuscher, F.; Li, D. L.; Diesel, A.; Ebel, R.; Proksch, P.; Wang, B. G. *J. Nat. Prod.* **2006**, *69*, 1622−1625.
(21) Crystallographic data of compounds **1** and **6** have been deposited in the Cambridge Crystallographic Data Centre as CCDC 801637 for compound **1** and CCDC 790228 for compound **6**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif (or from the CCDC, 12 Union Road, Cambridge CB21EZ, U.K.; fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
(22) Sheldrick, G. M. *SADABS, Software for Empirical Absorption Correction*; University of Göttingen: Germany, 1996.
(23) Sheldrick, G. M. *SHELXTL, Structure Determination Software Programs*: Bruker Analytical X-ray System Inc.: Madison, WI, 1997.
(24) Sheldrick, G. M. *SHELXL-97 and SHELXS-97, Program for X-ray Crystal Structure Solution and Refinement*; University of Göttingen: Germany, 1997.
(25) Bergeron, R. J.; Cavanaugh, P. F. Jr.; Kline, S. J.; Hughes, R. G. Jr.; Elliott, G. T.; Porter, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 848−854.
(26) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.
(27) Dore, M. P.; Osato, M. S.; Realdi, G.; Mura, I.; Graham, D. Y.; Sepulveda, A. R. *J. Antimicrob. Chemother.* **1999**, *43*, 47−54.
(28) Shen, C .C.; Syu, W. J.; Li, S. Y.; Lin, C, H.; Lee, G. H.; Sun, C. M. *J. Nat. Prod.* **2002**, *65*, 1857−1862.