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Aspewentins D−H, 20-Nor-isopimarane Derivatives from the Deep Sea Sediment-Derived Fungus *Aspergillus wentii* SD-310

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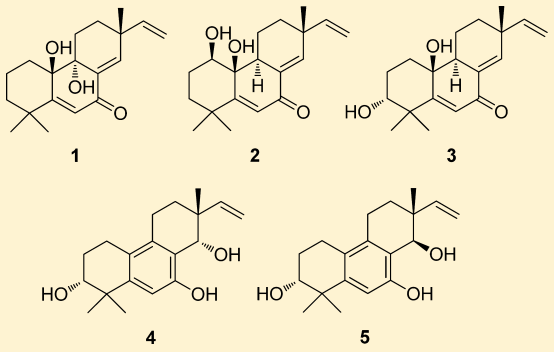
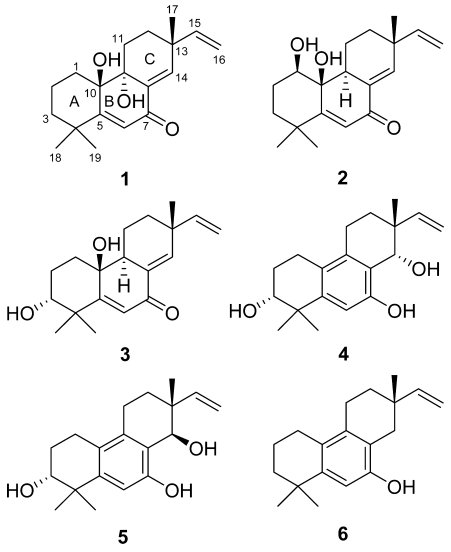
Supporting Information

ABSTRACT: Five new 20-nor-isopimarane diterpenoids, aspewentins D−H (**1**−**5**), along with a related known congener, aspewentin A (**6**), were isolated from the culture extract of *Aspergillus wentii* SD-310, a fungal strain obtained from a deep-sea sediment sample. The structures of these compounds were established on the basis of spectroscopic interpretation, and the absolute configurations of compounds **1**−**5** were determined by X-ray crystallographic analysis and TDDFT-ECD calculations. The isolated compounds were evaluated for antimicrobial activity against nine human and aquatic pathogenic bacteria and four plant pathogenic fungi as well as for lethality against brine shrimp (*Artemia salina*). 20-Nor-isopimarane derivatives rarely occur in fungi, and only three (aspewentins A−C) have previously been reported from a marine-derived fungus.



Microorganisms isolated from the deep sea have recently attracted considerable attention due to their potential for the discovery of pharmaceutically interesting metabolites.[1−5] As part of our efforts toward the chemical investigation of marine-derived fungi, a number of structurally interesting and biologically active compounds have been isolated and identified.[6−11] Recently, several tetranorditerpenoids such as asperolides A−C and wentilactones A and B were identified from static fermentation of the marine alga-derived endophytic fungus *Aspergillus wentii* EN-48,[11] and these compounds showed both *in vitro* and *in vivo* antitumor activities.[11−15] In the course of our continuing chemical investigation of marine-derived fungi, we recently obtained another strain of the same fungus, *A. wentii* SD-310, from a deep-sea sediment sample. This fungal strain displayed identical morphological characteristics to that of the previously reported algal-derived endophytic fungus *A. wentii* EN-48.[11] Chemical investigation of a culture extract of *A. wentii* SD-310 led to the isolation of five new 20-nor-isopimarane diterpenoids, namely, aspewentins D−H (**1**−**5**), along with the related known congener aspewentin A (**6**). The structures of these compounds were established by extensive analysis of spectroscopic data, and the absolute configurations of compounds **1**−**5** were determined by X-ray crystallographic analysis and quantum chemical ECD calculations. Compounds **1**−**6** were evaluated for antibacterial activities against two human and seven aquatic pathogenic bacteria and four plant pathogenic fungi as well as brine shrimp lethality against *Artemia salina*. This paper describes the isolation, structure elucidation, and bioactivities of compounds **1**−**6**.



RESULTS AND DISCUSSION

The mycelia and culture broth of *A. wentii* SD-310 were exhaustively extracted with MeOH and EtOAc, respectively, and the combined extracts were subjected to column chromatography (CC) on silica gel, Lobar LiChroprep RP-18, and

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Table 1. NMR Data for Compounds 1−3 in DMSO-d_6 (^{1}H at 500 MHz, ^{13}C at 125 MHz)

	1		2		3	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1α	29.8, CH_2	1.43, t (12.7)	67.7, CH	3.73, br s	29.9, CH_2	0.85, dd (7.0, 13.9)
1β		1.74, t (14.2)				1.80, m
2α	17.0, CH_2	1.84, m	24.2, CH_2	2.13, t (13.1)	24.7, CH_2	1.45, m
2β		1.48, m		1.46, m		2.07, t (12.7)
3α	39.1, CH_2	1.46, t (12.8)	32.0, CH_2	1.91, t (12.0)	74.4, CH	3.45, br s
3β		1.34, t (12.7)		1.18, d (13.3)		
4	36.3, C		35.5, C		41.6, C	
5	169.5, C		171.9, C		171.6, C	
6	124.2, CH	5.93, s	126.7, CH	6.02, s	126.1, CH	5.92, s
7	187.5, C		187.3, C		187.2, C	
8	135.6, C		133.6, C		133.1, C	
9	72.6, C		39.8, CH	2.95, t (6.7)	45.8, CH	2.38, t (6.9)
10	73.0, C		71.7, C		69.4, C	
11α	25.6, CH_2	1.53, br d (13.7)	17.0, CH_2	1.50, m	18.2, CH_2	1.73, m
11β		2.23, dd (11.2, 13.9)		1.82, m		1.85, m
12α	29.8, CH_2	1.94, dd (10.2, 13.3)	32.9, CH_2	1.78, m	33.3, CH_2	1.49, m
12β		1.71, m		1.60, d (13.3)		1.60, m
13	38.3, C		37.7, C		38.2, C	
14	143.2, CH	6.49, s	142.3, CH	6.55, s	142.3, CH	6.55, s
15	147.1, CH	5.89, dd (17.7, 10.6)	147.5, CH	5.87, dd (17.7, 10.4)	147.3, CH	5.86, dd (17.7, 10.4)
16	112.3, CH_2	5.01, br d (10.6)	112.1, CH_2	4.97, br d (10.4)	112.2, CH_2	4.96, br d (10.4)
		5.05, br d (17.7)		5.00, br d (17.7)		4.99, br d (17.7)
17	24.0, CH_3	1.05, s	25.4, CH_3	1.00, s	25.9, CH_3	1.09, s
18	30.2, CH_3	1.12, s	29.7, CH_3	1.16, s	26.3, CH_3	1.12, s
19	31.9, CH_3	1.21, s	32.0, CH_3	1.22, s	30.5, CH_3	1.18, s
1-OH				4.75, br s		
3-OH						4.56, br s
9-OH		4.83, s				
10-OH		4.81, s		4.78, br s		4.65, br s

Sephadex LH-20, as well as semipreparative HPLC, to yield compounds **1**−**6**.

Aspewentin D (**1**) was obtained as colorless crystals. Its molecular formula was assigned as $C_{19}H_{26}O_3$ by HRESIMS, with seven degrees of unsaturation. The ^{13}C NMR and DEPT data (Table 1) confirmed the presence of 19 carbon atoms: seven nonprotonated carbons, three olefinic methines, six methylenes (including one terminal olefinic methylene), and three methyls. Three methyl singlets (H-17 through H-19), five olefinic signals including one doublet of doublets (H-15), two doublets (H_2-16), and two singlets (H-6 and H-14), and two broad singlets characteristic of exchangeable protons (OH-9 and OH-10) were observed in the ^{1}H NMR spectrum (Table 1). Comparison of the NMR data with those reported for aspewentin C, a 20-nor-isopimarane diterpenoid from *A. wentii* na-3, which was isolated from the marine brown alga *Sargassum fusiforme*, indicated that compound **1** possesses the same carbon skeleton.[16] However, the double bond at C-8(9) in aspewentin C appeared at C-8(14) in **1**. These differences were corroborated by the fact that signals corresponding to two nonprotonated olefinic carbons at C-8(9) and the methylene group at C-14 in the NMR spectra of aspewentin C were absent in the data for **1**. Instead, resonances for two olefinic carbons, with one nonprotonated carbon at δ_C 135.6 (C-8) and one methine at δ_H 6.49/δ_C 143.2 (C-14) as well as one additional oxygenated, nonprotonated carbon at δ_C 72.6 (C-9), were present in the NMR spectra of **1** (Table 1). HMBC correlations from olefinic proton H-14 to C-7 and C-9 supported this deduction (Figure 1). Other HMBC and COSY correlations validated the planar structure of **1**.

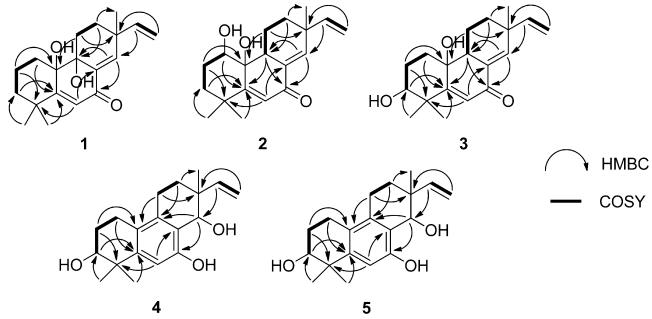

Figure 1. COSY (bold lines) and key HMBC (arrows) correlations of compounds **1**−**5**.

The relative configuration of **1** was assigned by analysis of its NOESY spectrum. The observed NOEs from the 9-OH to H-12α and from H-12α to H-15 located them on the same face of the molecule, while NOEs from the 10-OH to H-11β and from H-11β to H-17 assigned them on the other face of the molecule (Figure 2). The structure and relative configuration of **1** were confirmed by X-ray diffraction analysis (Figure 3). The final refinement of the data afforded a Flack parameter of 0.0(6), allowing unequivocal determination of the absolute configuration of **1** as 9*R*, 10*R*, and 13*R*. Besides the X-ray diffraction experiment, the electronic circular dichroism (ECD) spectrum of **1** was recorded and then computed with the time-dependent density function theory (TD-DFT) method at the gas-phase B3LYP/6-31G(d) level.[17,18] The calculated ECD spectra were produced by SpecDis software.[19] The experimental ECD

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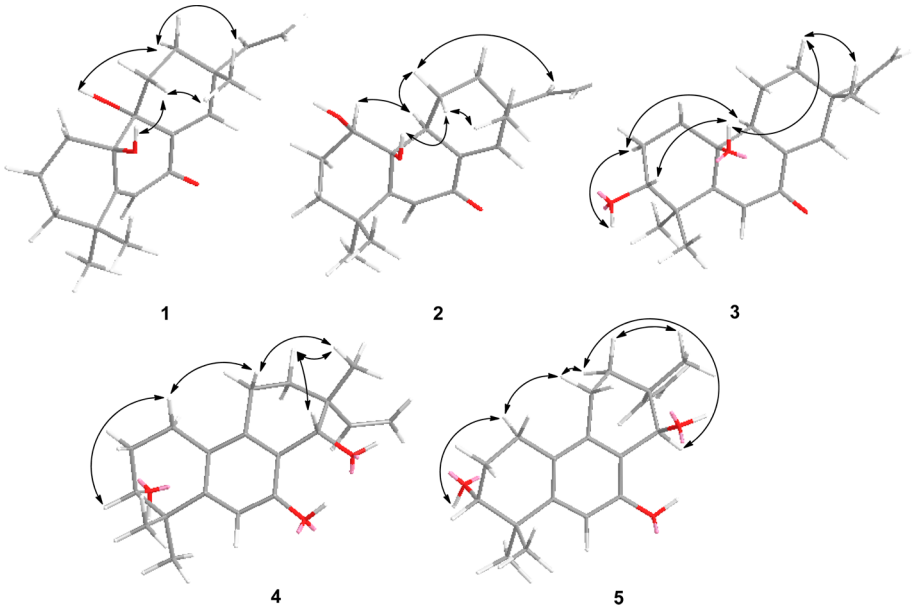

Figure 2. Key NOESY correlations of compounds **1**−**5**.

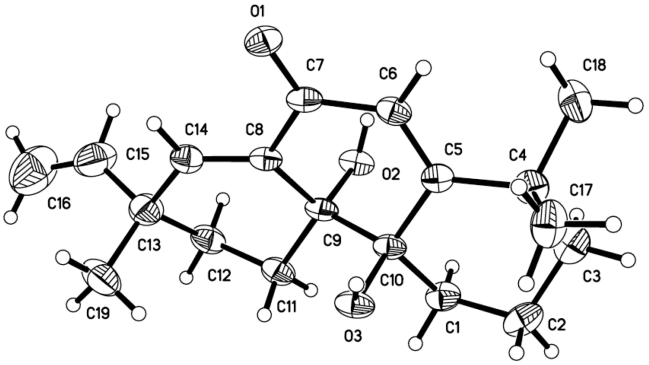

Figure 3. X-ray structure of compound **1**.

spectrum for **1** showed excellent agreement for the (9*R*, 10*R*, 13*R*) absolute configuration of **1** (Figure 4). Both the experimental and calculated data showed a positive Cotton effect (CE) near 236 nm and a negative CE around 268 nm.

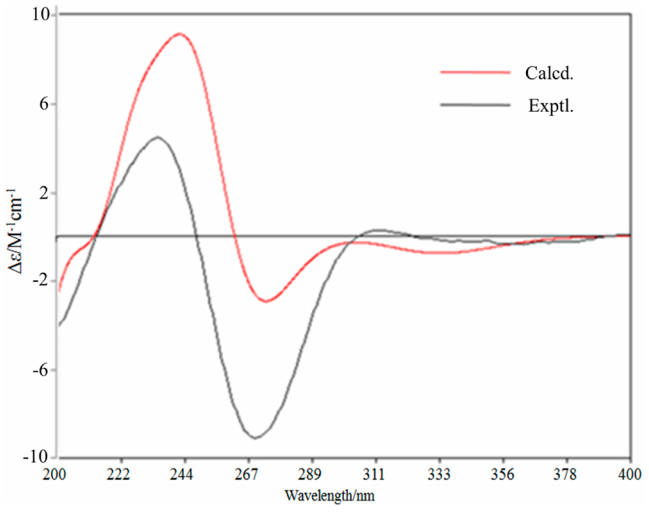

Figure 4. Experimental and calculated ECD spectra of compound **1**.

These close similarities confirmed the absolute configuration for **1** as shown.

Aspewentin E (**2**), obtained as a white, amorphous powder, was assigned the same molecular formula as **1** on the basis of HRESIMS data. Analysis of the 1D NMR data (Table 1) indicated that compound **2** is also a 20-nor-isopimarane diterpenoid. However, comparison of the NMR data revealed that the OH group at C-9 of **1** was absent in the structure of **2** and was replaced by an OH group at C-1. COSY correlations from H-1 to H_2-2 and from H-9 to H_2-11 as well as HMBC correlations from H-1 to C-5 and from H-9 to C-5 and C-7 (Figure 1) supported the above deduction. The relative configuration of **2** was again deduced from the NOESY data. Correlations from H-1 to H-9, from H-9 to H-11α, and from H-11α to H-15 placed these hydrogens on the same face of the molecule, while NOEs from the 10-OH to H-11β and from H-11β to H_3-17 located them on the other face of the molecule (Figure 2).

The ECD spectrum of **2** was very similar to that of **1**, suggesting that **2** has the same absolute configuration as that of **1**. The experimental spectrum for **2** again matched well for the (1*R*, 9*S*, 10*S*, 13*R*) isomer (Figure S16, Supporting Information). Both of the experimental and calculated data showed a positive CE near 231 nm and a negative CE around 268 nm. These close similarities allowed assignment of the absolute configuration of **2** as shown.

Aspewentin F (**3**) was an isomer of **1** and **2** as established by HRESIMS data. Examination of the ^{1}H, ^{13}C, and DEPT NMR data of **3** (Table 1) and comparison with those of **1** and **2** revealed that the structure of **3** was similar to that of **2** except for some variations of resonances corresponding to the C-1 through C-4 portion of the molecule. The 1D NMR spectroscopic data (Table 1) differed from those of **2** mainly in the absence of the signal for the OH at C-1, whereas resonances corresponding to an oxygenated CH unit at δ_H 3.45 (H-3) and δ_C 74.4 (C-3) were present in the NMR spectra of **3**. COSY correlations from H-2 to H-1 and H-3 and HMBC correlations from H-3 to C-1, C-5, and C-18 (Figure 1) supported the above deduction. NOE correlations from OH-10 to H-3 and H-12β and from H-12β to H-17 arranged these groups on the same face of the molecule, while NOEs from the 3-OH to H-2α and from H-2α to H-9

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located them on the other face of the molecule (Figure 2). The ECD spectrum of **3** (Figure S24) was very similar to those of **1** and **2** and matched well with that calculated for (3*R*,9*S*,10*S*,13*R*)-**3**. The structure and absolute configuration of **3** were thus assigned as shown.

Aspewentin G (**4**) was obtained as a white, amorphous powder. Its molecular formula was also established as $C_{19}H_{26}O_3$ on the basis of HRESIMS data, indicating that it is an isomer of compounds **1**−**3**. However, the NMR spectra of **4** are much different from those of **1**−**3**. Comparison of the NMR data with those reported for aspewentin A (**6**), an aromatic norditerpenoid,[16] revealed that **4** might be a 3,14-dihydroxylated derivative of **6**. The 1D NMR spectroscopic data of **4** (Table 2) differed

Table 2. NMR Data for Compounds 4 and 5 in DMSO-d_6 (^{1}H at 500 MHz, ^{13}C at 125 MHz)

	4		5	
position	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1α	24.3, CH_2	2.42, m	23.9, CH_2	2.57, m
1β		2.51, m		2.26, m
2α	27.5, CH_2	1.71, m	27.5, CH_2	1.71, m
2β		1.81, m		1.82, m
3	73.7, CH	3.43, br d (10.0)	73.4, CH	3.45, br d (9.2)
4	39.2, C		39.1, C	
5	144.9, C		144.7, C	
6	111.1, CH	6.63, s	110.9, CH	6.63, s
7	154.5, C		154.4, C	
8	123.1, C		123.1, C	
9	134.1, C		134.5, C	
10	123.2, C		123.6, C	
11α	23.9, CH_2	2.36, m	24.2, CH_2	2.47, m
11β		2.33, m		2.29, m
12α	27.3, CH_2	1.96, m	28.7, CH_2	1.87, m
12β		1.43, dd (5.6, 12.5)		1.51, m
13	39.2, C		39.4, C	
14	69.0, CH	4.36, br s	68.2, CH	4.45, br s
15	147.2, CH	6.10, dd (17.7, 10.9)	144.9, CH	5.65, dd (17.7, 10.8)
16	111.7, CH_2	4.96, br d (10.9)	112.9, CH_2	4.86, br d (10.8)
		5.00, br d (17.7)		4.94, br d (17.7)
17	21.4, CH_3	0.79, s	24.0, CH_3	1.04, s
18	25.5, CH_3	1.04, s	25.8, CH_3	1.07, s
19	29.2, CH_3	1.18, s	29.0, CH_3	1.23, s
3-OH		4.61, d (4.0)		4.57, br s
7-OH		8.99, br s		9.03, br s
14-OH		4.52, br s		4.68, br s

from those of **6** mainly in the absence of signals for two CH_2 units at δ_H 1.60/δ_C 38.7 (CH_2-3) and δ_H 2.44/2.64/δ_C 34.4 (CH_2-14). Instead, resonances corresponding to two oxymethines at δ_H 3.43/δ_C 73.7 (CH-3) and δ_H 4.36/δ_C 69.0 (CH-14) were present in the NMR spectra of **4**. COSY correlations from H-2 to H-1 and H-3 and HMBC correlations from H-3 to C-5, from H-18 to C-3, from H-14 to C-7 and C-9, and from H-15 to C-14 (Figure 1) supported the above deduction. NOE correlations from H-3 to H-1β, from H-1β to H-11β, from H-11β to H-17, from H-17 to H-12β, and from H-12β to H-14 suggested H-3, H-14, and H-17 were located on the same face of the molecule (Figure 2). The experimental ECD spectrum for **4** showed excellent agreement with that calculated for (3*R*,13*R*,14*S*)-**4** (Figure 5). These close similarities allowed assignment of the absolute configuration for **4** as shown.

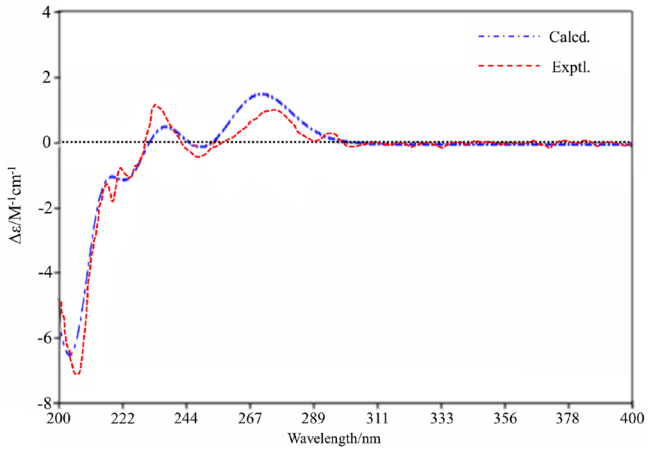

Figure 5. Experimental and calculated ECD spectra of compound **4**.

Aspewentin H (**5**) was also obtained as a white, amorphous powder. A molecular formula of $C_{19}H_{26}O_3$ was established on the basis of HRESIMS data, indicating that **5** is an isomer of compounds **1**−**4**. The NMR data of **5** showed almost identical patterns to those of **4**, with some minor variations for the chemical shifts of C-13 through C-17. Inspection of the ^{1}H and ^{13}C NMR data suggested that **5** is a diastereomer of **4**, epimeric at C-14. This was supported by the observed NOEs from H-14 to H-12α, from H-12α to H-11α, from H-11α to H-1α, and from H-1α to 3-OH, as well as from H-12β to H-17 (Figure 2). The absolute configuration of compound **5** was also established by comparing the calculated ECD spectrum with that of the experimental data. As a result, the experimental ECD spectrum of **5** showed excellent accordance with (3*R*,13*R*,14*R*)-**5** (Figure S40).

In addition to the new compounds **1**−**5**, the known 20-norisopimarane diterpenoid aspewentin A (**6**) was also isolated, and its structure was determined by comparing the spectroscopic data with those reported in the literature.[16] Several 20-norisopimarane diterpenoids including smardaesidins F and G from the moss endophytic fungus *Smardaea* sp. AZ0432,[20] diplopimarane from the oak pathogen *Diplodia quercivora*,[21] and xylopimarane, the glucoside derivative of diplopimarane, from the dead-wood-derived fungus *Xylaria* sp. BCC 4297[22] have been described from terrestrial sources, while only one paper has described the isolation of such compounds, aspewentins A−C, from a marine alga-derived isolate of the fungus *A. wentii*.[16]

Compounds **1**−**6** were evaluated for antimicrobial activity against two human pathogenic bacteria, seven aquatic pathogens, and four plant pathogenic fungi (Table 3). Compounds **1** and **3**−**6** showed inhibitory activity against the aquatic pathogens *Edwardsiella tarda*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Vibrio harveyi*, and *V. parahemolyticus*, each with MIC values of 4.0 μg/mL, while compounds **1** and **5** showed activity against the plant pathogen *Fusarium graminearum*, with MIC values of 2.0 and 4.0 μg/mL, respectively, which are more potent than that of the positive control amphotericin B (MIC 8.0 μg/mL). Compound **1** was more active toward bacteria and fungi than **2** and **3**, suggesting that compounds with OH substitution at C-9 are more active than those with OH substitution at C-1 or C-3. Furthermore, compounds with an aromatized ring B were more active (**4** and **5**) than that of nonaromatic derivatives **2** and **3**.

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Table 3. Antimicrobial Activities of Compounds 1−6 (MIC, μg/mL)[a]

strain	compound						
	1	2	3	4	5	6	positive control
E. coli[b]	32	–	–	16	32	–	4.0
S. aureus[b]	–	–	32	32	–	8.0	0.5
A. hydrophilia[b]	–	–	16	–	–	–	4.0
E. tarda[b]	–	–	4.0	–	–	16	8.0
M. luteus[b]	4.0	16	–	–	16	–	8.0
P. aeruginosa[b]	32	–	–	–	4.0	–	4.0
V. anguillarum[b]	–	–	32	–	32	–	0.5
V. harveyi[b]	–	–	8.0	4.0	–	–	4.0
V. parahemolyticus[b]	–	32	32	–	32	4.0	1.0
A. brassicae[c]	8.0	32	–	–	32	–	32
C. gloeosprioides[c]	–	–	–	32	32	–	32
F. graminearum[c]	2.0	–	–	–	4.0	–	8.0
G. graminis[c]	32	–	–	32	32	8.0	64

[a](−) = MIC > 32 μg/mL. [b]Chloramphenicol as positive control. [c]Amphotericin B as positive control.

Compounds **1**−**6** were also evaluated for brine shrimp lethality against *Artemia salina*, but none of them displayed significant activity (LD_{50} > 10 μg/mL).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined with an SGW X-4 micromelting-point apparatus. Optical rotations were measured on an Optical Activity AA-55 polarimeter. UV spectra were measured on a PuXi TU-1810 UV−visible spectrophotometer. ECD spectra were acquired on a Chirascan spectropolarimeter. 1D and 2D NMR spectra were recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance 500 MHz spectrometer with tetramethylsilane as internal standard. Mass spectra were obtained on a VG Autospec 3000 or an API QSTAR Pulsar 1 mass spectrometer. Analytical and semipreparative HPLC were performed using a Dionex HPLC system equipped with a P680 pump, an ASI-100 automated sample injector, and a UVD340U multiple-wavelength detector controlled by Chromeleon software (version 6.80). 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. All solvents were distilled prior to use.

Fungal Material. *Aspergillus wentii* SD-310 was isolated from a deep-sea sediment sample collected in the South China Sea at a depth of 2038 m in May 2012. The fungus was identified using a molecular biological protocol by DNA amplification and sequencing of the ITS region, as described in our previous report.[23] The sequence data derived from the fungal strain have been deposited at GenBank (accession no. KM409566). A BLAST search result showed that the sequence was the same (100%) as that of *Aspergillus wentii* EN-48 (accession no. HM014129.1) and *Aspergillus wentii* na-3 (accession no. KF921087.1). The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences (IOCAS).

Fermentation, Extraction, and Isolation. For chemical investigations, the fungal strain was dynamically fermented in a 500 L fermentor for 7 days at room temperature preloaded with 300 L of sterilized liquid medium (20% potato, 2% glucose, 0.5% peptone, and 0.3% yeast extract, pH 6.0) containing 50% (v/v) seawater collected from the Hui Quan Bay near the campus of IOCAS.

The whole fermented cultures were filtered to separate the broth from the mycelia. The former was extracted three times with EtOAc, while the latter was extracted three times with a mixture of acetone and H_2O (80:20, v/v). The acetone solution was evaporated under reduced pressure to afford an aqueous solution, which was then extracted three times with EtOAc. Because the TLC and HPLC profiles of the two EtOAc solutions from the broth and mycelia were almost identical, they were combined and concentrated under reduced pressure to give an extract (34.7 g) for further separation.

The organic extract was fractionated by vacuum liquid chromatography (VLC) on silica gel eluting with different solvents of increasing polarity from petroleum ether (PE) to MeOH to yield 10 fractions (Frs. 1−10), which were pooled based on TLC analysis. Frs. 1 and 2 (18 g) were found to mainly consist of aliphatic compounds, mostly fatty acids, and were not further processed. Fr. 3 (5.1 g), eluted with PE−EtOAc (5:1), was further purified by CC on Sephadex LH-20 (MeOH) to afford two subfractions (Fr. 3-1 and Fr. 3-2). Fr. 3-1 was further purified by CC over RP-18 eluting with a MeOH−H_2O gradient (from 1:9 to 1:0) and by semipreparative HPLC (Elite ODS-BP column, 10 μm; 10.0 × 300 mm; MeOH−H_2O, from 85% to 90%, flow rate 3 mL/min) to afford compounds **2** (10.3 mg, t_R 18.8 min) and **3** (9.7 mg, t_R 17.5 min). Fr. 3-2 was further purified by Sephadex LH-20 (MeOH) and by semipreparative HPLC (90% MeOH−H_2O, 3 mL/min) to afford **1** (16.8 mg, t_R 20.1 min). Fr. 4 (4.5 g), eluted with $CHCl_3$−MeOH (20:1), was further purified by CC on silica gel, eluting with a PE−acetone gradient (from 10:1 to 1:1), and by semipreparative HPLC (85% MeOH−H_2O, 3 mL/min) to afford **4** (6.4 mg, t_R 17.5 min) and **6** (7.0 mg, t_R 16.1 min). Fr. 5 (4.2 g), eluted with $CHCl_3$−MeOH (10:1), was purified by CC on silica gel eluting with a $CHCl_3$−MeOH gradient (30:1 to 10:1) and further purified by CC over RP-18, eluting with a MeOH−H_2O gradient (1:9 to 1:0), and by semipreparative HPLC (MeOH−H_2O, 80% to 85%, 3 mL/min) to obtain **5** (8.1 mg, t_R 18.8 min).

*Aspewentin D (**1**):* colorless single crystal (MeOH); mp 222−224 °C; $[\alpha]_D^{20}$ −109 (*c* 0.90, MeOH); UV (MeOH) λ_{max} (log ε) 205 (−1.73), 260 (3.02) nm; ECD (0.38 mM, MeOH) λ_{max} (Δε) 236 (+4.39), 268 (−8.69), 356 (−0.22) nm; IR (KBr) ν_{max} 3368, 2958, 2925, 2865, 1662, 1636, 1458, 1403, 1313, 1142, 962, 911 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; ESIMS *m*/*z* 303 $[M + H]^+$; HRESIMS *m*/*z* 303.1950 $[M + H]^+$ (calcd for $C_{19}H_{27}O_3$, 303.1955).

*Aspewentin E (**2**):* white, amorphous powder; $[\alpha]_D^{20}$ −112 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 200 (2.05), 260 (2.20) nm; ECD (0.20 mM, MeOH) λ_{max} (Δε) 231 (+2.51), 268 (−4.29) nm; IR (KBr) ν_{max} 3336, 2922, 2854, 1662, 1601, 1454, 1384, 1297, 1069, 1003, 913 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; ESIMS *m*/*z* 303 $[M + H]^+$; HRESIMS *m*/*z* 303.1962 $[M + H]^+$ (calcd for $C_{19}H_{27}O_3$, 303.1955).

*Aspewentin F (**3**):* white, amorphous powder; $[\alpha]_D^{20}$ −73 (*c* 1.21, MeOH); UV (MeOH) λ_{max} (log ε) 200 (2.30), 265 (2.81) nm; ECD (0.34 mM, MeOH) λ_{max} (Δε) 233 (+3.59), 265 (−6.89), 358 (−0.24) nm; IR (KBr) ν_{max} 3350, 2952, 2926, 2862, 1661, 1621, 1454, 1334, 1290, 1030, 999, 985 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; ESIMS *m*/*z* 303 $[M + H]^+$; HRESIMS *m*/*z* 303.1960 $[M + H]^+$ (calcd for $C_{19}H_{27}O_3$, 303.1955).

*Aspewentin G (**4**):* white, amorphous powder; $[\alpha]_D^{20}$ −6.7 (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.05), 285 (0.21) nm; ECD (0.24 mM, MeOH) λ_{max} (Δε) 205 (−6.87), 220 (−1.05), 224 (−1.86),

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240 (+1.09), 253 (−0.21), 271 (+1.07) nm; IR (KBr) ν_{max} 3332, 2929, 2869, 1601, 1418, 1317, 1259, 1020, 1003, 912 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; ESIMS m/z 301 [M − H]$^-$; HRESIMS m/z 301.1804 [M − H]$^-$ (calcd for $C_{19}H_{25}O_3$, 301.1798).

Aspewentin H (5): white, amorphous powder; $[\alpha]_D^{20}$ +4.0 (c 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.25), 285 (0.22) nm; ECD (0.21 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 209 (+2.01), 233 (−0.91), 255 (+2.07) nm; IR (KBr) ν_{max} 3351, 2931, 2254, 2126, 1603, 1420, 1319, 1259, 1023, 1004, 915, 863, 823, 761 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 2; ESIMS m/z 301 [M − H]$^-$; HRESIMS m/z 301.1803 [M − H]$^-$ (calcd for $C_{19}H_{25}O_3$, 301.1798).

X-ray Crystallographic Analysis.[24] A colorless crystal of compound **1** was obtained from a solution of MeOH. Crystallographic data were collected on a Srigaku Mercury CCD/AFCR diffractometer equipped with graphite-monochromatic Cu Kα radiation (λ = 1.541 78 Å) at 293(2) K. The data were corrected for absorption by using the program SADABS.[25] The structure was solved by direct methods and subsequent difference Fourier synthesis and refined by full-matrix least-squares techniques with the SHELXTL software package.[26] All non-hydrogen atoms were refined anisotropically. The H atoms belonging to C atoms were calculated theoretically, and those to O atoms were determined by difference Fourier maps.[27]

Crystal data of 1: $C_{19}H_{26}O_3$; fw = 302.40, monoclinic space group $P2(1)$, unit cell dimensions a = 5.9126(3) Å, b = 22.9872(19) Å, c = 12.2565(8) Å, V = 1665.76(19) Å^3, $\alpha = \gamma = 90°$, β = 90.5130(10)°, Z = 8, d_{calcd} = 1.206 mg/m^3, crystal dimensions 0.27 × 0.24 × 0.14 mm, μ = 0.663 mm^{-1}, $F(000)$ = 656. The 9368 measurements yielded 5338 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R_1 = 0.0838 and wR_2 = 0.2491 [$I > 2\sigma(I)$]. The Flack parameter was 0.0(6) in the final refinement for all 9368 reflections with 5338 Friedel pairs.

Antimicrobial Assays. Antimicrobial evaluation against two pathogenic bacteria (*Escherichia coli* EMBLC-1 and *Staphyloccocus aureus* EMBLC-2) and seven aquatic pathogens (*Aeromonas hydrophilia* QDIO-1, *E. tarda* QDIO-2, *M. luteus* QDIO-3, *P. aeruginosa* QDIO-4, *V. anguillarum* QDIO-6, *V. harveyi* QDIO-7, and *V. parahemolyticus* QDIO-8) as well as four plant pathogenic fungi (*Alternaria brassicae* QDAU-1, *Colletotrichum gloeosprioides* QDAU-2, *F. graminearum* QDAU-4, and *Gaeumannomyces graminis* QDAU-3) was carried out by the microplate assay.[28] The pathogenic bacteria and aquatic pathogenic strains were provided by the Institute of Oceanology, Chinese Academy of Sciences, while the plant pathogenic fungal strains were obtained from Qingdao Agricultural University. Chloramphenicol and amphotericin B were used as positive controls against bacteria and fungi, respectively.

Brine Shrimp Toxicity Assay. Evaluation of brine shrimp lethality against *Artemia salina* was performed as previously reported.[29]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b01153.

Crystallographic data (CIF)

Selected 1D and 2D NMR and ECD spectra of compounds **1**−**5** (PDF)

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Notes

The authors declare no competing financial interest.

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