

Article

Three New Indole Diterpenoids from the Sea-Anemone-Derived Fungus *Penicillium* sp. AS-79

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Abstract: Three new indolediterpenoids, namely, 22-hydroxylshearinine F (**1**), 6-hydroxylpaspalinine (**2**), and 7-*O*-acetylemindole SB (**3**), along with eight related known analogs (**4–11**), were isolated from the sea-anemone-derived fungus *Penicillium* sp. AS-79. The structures and relative configurations of these compounds were determined by a detailed interpretation of the spectroscopic data, and their absolute configurations were determined by ECD calculations (**1** and **2**) and single-crystal X-ray diffraction (**3**). Some of these compounds exhibited prominent activity against aquatic and human pathogenic microbes.

Keywords: endophytic fungus; *Penicillium* sp.; secondary metabolites; antimicrobial activity

1. Introduction

Indolediterpenoids (IDTs) are a group of fungal metabolites that have attracted the attention of natural product chemists due to their diversified structures and potent biological activities [1,2]. IDTs usually possess a common core structure comprised of an indole moiety and is most likely derived from indole-3-glycerol phosphate and a geranyldiphosphate-derived cyclic diterpenoid skeleton [3]. The typical IDTs reported from fungal sources are penitrems, janthitrems, lotitrems, aflatrems, paxilline, and shearinines [2]. Previous reports revealed that IDTs possessed various bioactivities including antibiotic [4,5] and anti-insectan potency [6,7] as well as tremorgenic mammalian mycotoxicity [8,9]. During our investigation on marine-derived fungi, a series of structurally interesting secondary metabolites such as cyclohexadepsipeptides [10], diketopiperazine alkaloids [11], and diphenylketones [12] were discovered. Recently, we focused our attention on *Penicillium* sp. AS-79, a fungal strain isolated from the fresh tissue of the sea anemone *Haliplanella luciae*, and, as a result, three new (**1–3**) and eight known (**4–11**) indolediterpenoids are isolated and elucidated (Figure 1). Details of the structure determination and biological activities of these compounds are presented herein.

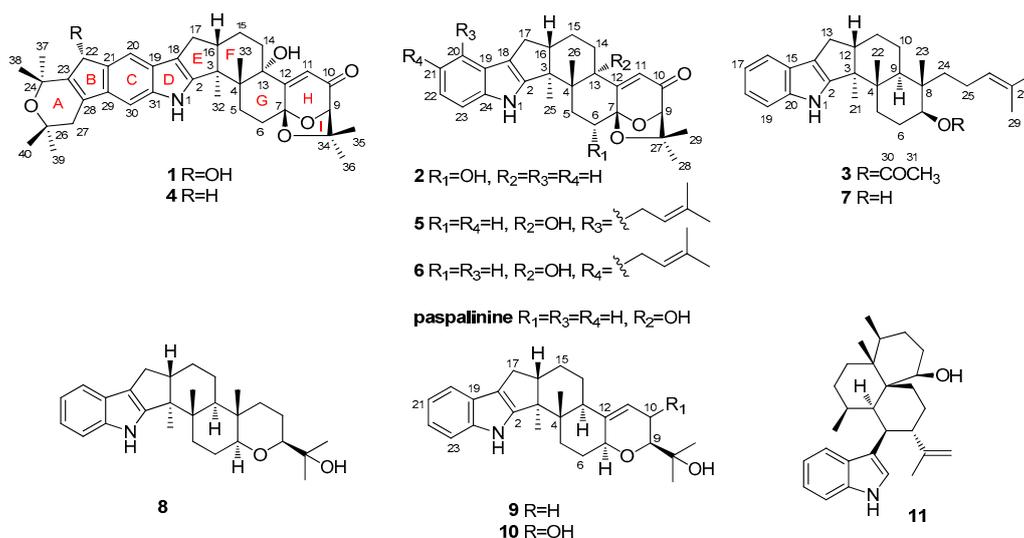


Figure 1. Structures of 1–11 and paspalinine.

2. Results and Discussion

2.1. Structure Elucidation of the New Compounds

Compound **1** was obtained as a colorless solid. Its molecular formula was determined as C₃₇H₄₅NO₆ on the basis of (+)-HRESIMS data, with 16 degrees of unsaturation. The ¹H NMR data (Table 1) revealed the presence of eight methyl singlets and six methines (including three aromatic/olefinic and two oxygenated) as well as six methylenes in **1**. Its ¹³C NMR and DEPT data (Table 1) showed the presence of 37 carbon resonances, including eight methyls, six sp³ methylenes, six methines (including three aromatic/olefinic, two oxygenated and/or heteroatom-bonded, and one sp³ methine), and 17 quaternary carbons. The ¹H and ¹³C data of **1** are very similar to those of shearinine F (**4**), an indole diterpenoid isolated from the marine mangrove-derived endophytic fungus *Penicillium* sp. (strain HKI0459) [13], indicating that **1** is also an indole diterpenoid derivative. Detailed comparison of the NMR data between **1** and **4** revealed that the methylene group at δ_C 36.2/δ_H 3.38 (CH₂-22) of **4** was replaced by an oxygenated methine group at δ_C 76.3/δ_H 5.19 (CH-22) (Table 1). The observed HMBC correlations from H-22 to C-20 and C-24 confirmed the replacement of C-22 methylene in **4** by oxymethine in **1** (Figure 2).

Table 1. ¹H (125 MHz) and ¹³C NMR (500 MHz) data of Compounds 1–3 (δ in ppm, J in Hz).

No.	1 ^a		2 ^a		3 ^b	
	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C
1	7.75, s		7.73, s		9.79, s	
2		151.8, C		149.9, C		151.8, C
3		51.8, C		51.2, C		53.9, C
4		40.1, C		38.1, C		38.6, C
5	α 2.79, m β 1.72, m	27.2, CH ₂	α 2.59, d (14.8) β 2.17, d (5.2)	37.5, CH ₂	α 1.80, m β 1.73, m	24.7, CH ₂
6	α 2.82, m β 2.05, m	28.4, CH ₂	4.15, d (5.0)	70.4, CH	α 1.89, m β 1.69, m	23.3, CH ₂
7		104.5, C		103.3, C	4.88, dd (10.7, 4.3)	75.9, CH
8						40.0, C
9	4.33, s	88.2, CH	4.37, s	88.4, CH	1.51, br.s	41.0, CH

Table 1. Cont.

No.	1 ^a		2 ^a		3 ^b	
	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C
10		197.2, C		195.2, C	α 1.79, m β 1.72, m	28.0, CH ₂
11	5.86, s	117.8, CH	5.87, s	118.7, CH	α 1.80, m β 1.68, m	33.1, CH ₂
12		169.8, C		171.3, C	2.80, m	49.8, CH
13		77.8, C	3.68, dt (3.4, 11.7)	39.5, CH	α 2.36, dd (13.1, 10.9) β 2.68, dd (13.1, 6.4)	28.0, CH ₂
14	α 2.05, m β 1.92, m	34.5, CH ₂	α 1.97, m β 1.48, m	24.1, CH ₂		117.9, C
15	α 2.05, m β 1.72, m	21.3, CH ₂	α 1.85, m β 1.77, m	24.0, CH ₂		131.5, C
16	2.64, m	48.6, CH	2.83, m	48.4, CH	7.36, d (7.4)	118.8, CH
17	α 2.79, dd (12.4, 5.6) β 2.05, dd (12.4, 3.1)	27.8, CH ₂	α 2.74, dd (13.2, 6.3) β 2.41, dd (13.2, 10.6)	27.8, CH ₂	6.99, dt (7.1, 1.6)	119.7, CH
18		117.8, C		118.5, C	6.99, dt (7.1, 1.6)	120.6, CH
19		123.6, C		125.3, C	7.32, d (7.3)	112.6, C
20	7.56, s	114.1, CH	7.46, d (7.9)	119.9, CH	7.32, d (7.3)	141.7, C
21		136.8, C	7.11, t (6.2)	120.8, CH	1.10, s	15.0, CH ₃
22	5.19, s	76.3, CH	7.11, t (6.2)	121.5, CH	1.18, s	19.4, CH ₃
23		144.8, C	7.34, d (8.2)	111.7, CH	0.95, s	18.1, CH ₃
24		73.8, C		140.3, C	α 1.52, m β 1.23, m	29.9, CH ₂
25			1.16, s	15.5, CH ₃	α 2.08, m β 1.80, m	22.1, CH ₂
26		71.3, C	1.18, s	21.6, CH ₃	5.11, t (7.1)	125.5, CH
27	α 2.45, d (18.0) β 2.43, d (18.0)	34.1, CH ₂		79.8, C		126.1, C
28		138.4, C	1.44, s	28.7, CH ₃	1.63, s	25.9, CH ₃
29		133.9, C	1.24, s	23.2, CH ₃	1.69, s	17.6, CH ₃
30	7.02, s	102.1, CH				170.6, C
31		140.0, C			2.05, s	21.1, CH ₃
32	1.37, s	16.3, CH ₃				
33	1.25, s	23.8, CH ₃				
34		78.9, C				
35	1.20, s	23.2, CH ₃				
36	1.46, s	29.0, CH ₃				
37	1.55, s	30.4, CH ₃				
38	1.50, s	30.0, CH ₃				
39	1.29, s	29.7, CH ₃				
40	1.37, s	29.5, CH ₃				

^a Measured in CDCl₃. ^b Measured in acetone-*d*₆.

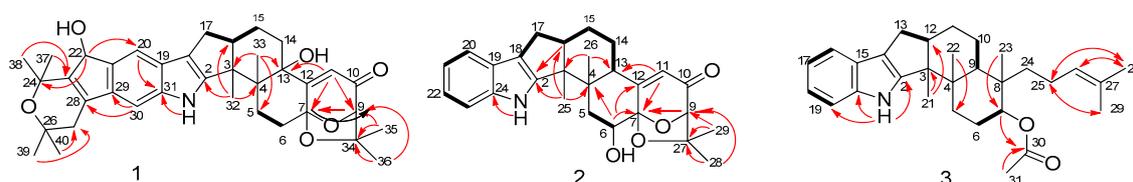


Figure 2. Key COSY (bold line) and HMBC (arrow line) correlations of Compounds 1–3.

The relative configuration of **1** was determined by the NOESY spectrum. The NOESY correlations from H-32 to H-17 α , from H-14 β to H-16 and H-33, and from H-14 α to H-11 confirmed the *trans* fusion of Rings E and F and of Rings F and G. For Rings A and B, the NOESY correlation between H-22 and H-38 placed the OH group on C-22 and CH₃-38 on the opposite face (Figure 3).

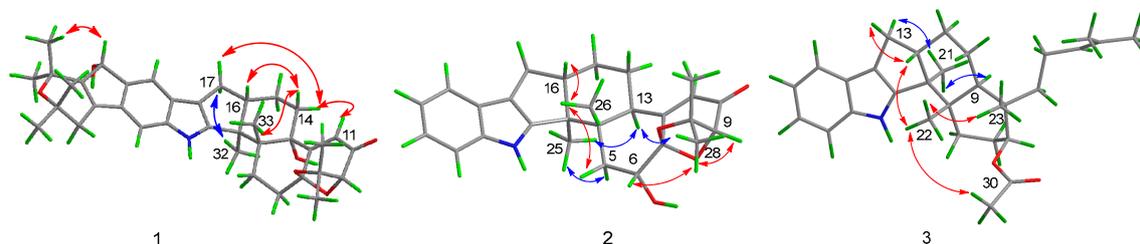


Figure 3. NOESY correlations of Compounds 1–3.

To determine the absolute configurations of the stereogenic carbons and of the compound, conformational search, geometry optimization of each possible isomer, and time-dependent density functional (TDDFT)-ECD calculation were performed using Gaussian 09.10. The obtained minimum energy conformers were calculated for their ECD spectra by the TDDFT method at the B3LYP/6-31G(d) level. The experimental ECD spectrum of **1** matched well with that calculated for 3*S*, 4*R*, 7*S*, 9*R*, 13*S*, 16*S*, and 22*R* configurations, which showed positive cotton effects (CEs) at approximately 355 and 272 nm and a negative CE near 237 nm (Figure 4). Thus, the structure of **1** was determined and was named 22-hydroxylshearinine F.

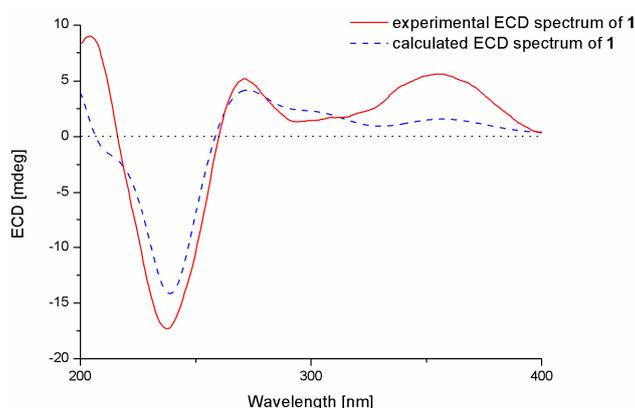


Figure 4. Experimental and calculated ECD spectra of **1**.

Compound **2** was obtained as a colorless solid. Its molecular formula was determined as C₂₇H₃₁NO₄ on the basis of (+)-HRESIMS, indicating 13 degrees of unsaturation. The ¹H and ¹³C NMR spectra of **2** are very similar to those of paspalinine (Figure 1), an indolediterpenoid isolated from the marine mangrove-derived endophytic fungus *Alternariatenuissima* EN-192 [14]. However, inspection of the NMR data revealed that CH₂-6 (δ_C 29.5; δ_H 2.41/2.64) in paspaline was replaced by an oxymethine group (δ_C 70.4; δ_H 4.15) (Table 1). This was further supported by the observed COSY correlation between H-5 and H-6 as well as by the HMBC correlations from H-6 to C-4, C-7, and C-12 (Figure 2).

The relative configurations of the stereogenic carbons of **2** were determined by NOESY spectrum. The NOESY correlations from H-26 to H-5 β and H-16 indicated the same orientation of these protons, while the correlations from H-25 to H-5 α and H-13 and from H-13 to H-28 as well as from H-28 to H-6 and H-9 indicated the opposite orientation of these groups (Figure 3). The absolute configurations of the stereogenic carbons of **2** were also determined by the TDDFT-ECD calculation in Gaussian 09.10. The experimental ECD spectrum of **2** showed excellent agreement with that calculated for 3*S*, 4*S*, 6*R*,

7*R*, 9*R*, 13*R*, and 16*S* configurations (Figure 5), and the structure of **2** was thus established and was named 6-hydroxylpaspalinine.

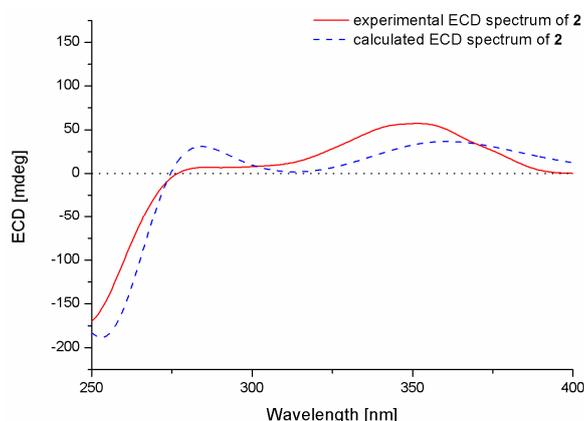


Figure 5. Experimental and calculated ECD spectra of **2**.

Compound **3** was obtained as colorless crystals. Its molecular formula was determined as $C_{30}H_{41}NO_2$ on the basis of (+)-HRESIMS data, indicating 11 degrees of unsaturation. Except for the presence of the acetyl group (δ_C 170.6; δ_C 21.1/ δ_H 2.05, s), the NMR data of **3** are very similar to those of emindole SB (**7**), an indolediterpenoid isolated from the marine mangrove-derived endophytic fungus *Dichotomomyces cepii* [15], indicating that **3** is an acetylated derivative of **7**.

The relative configurations of the stereogenic carbons of **3** were determined by NOESY spectrum. The NOESY correlations from H-21 to H-9 and H-13 α indicated the same orientation of these protons, while correlations from H-22 to H-12, H-23, and H-31 as well as from H-12 to H-13 β indicated the other orientation of these protons (Figure 3). X-ray analysis confirmed the structure of **3** and determined the absolute configurations of C-3, 4, 7, 8, and 9 as 3*S*, 4*S*, 7*S*, 8*S*, and 9*S*. The ortep view of **3** is shown in Figure 6. Compound **3** was named 7-*O*-acetylemindole SB.

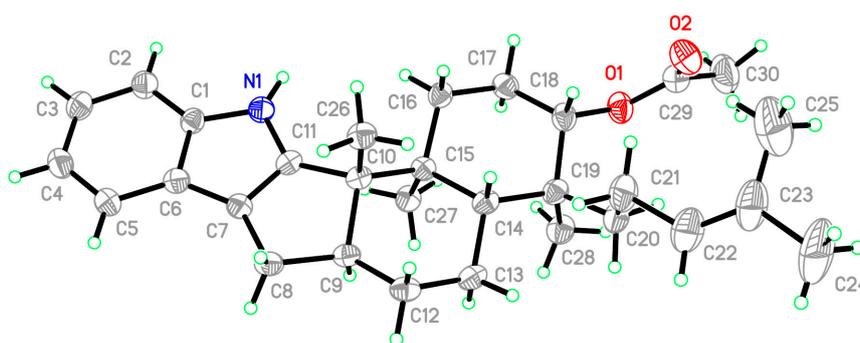


Figure 6. X-ray structure of Compound **3** (note: a different numbering system is used for the structure in the text).

In addition to the isolation of Compounds **1–3**, eight other congeners—shearinine F (**4**) [13], paspalitrem C (**5**) [16], paspalitrem A (**6**) [16], emindole SB (**7**) [15], paspaline (**8**) [17], 3-deoxo-4b-deoxyaxilline (**9**) [18], PC-M6 (**10**) [19], and 10,23-dihydro-24,25-dehydroaflavinine (**11**) [20]—were also isolated and identified. Their structures were determined by analysis of the spectroscopic data and by comparison with that reported in the literature.

2.2. Biological Activities of the Isolated Compounds

The isolated compounds (1–11) were examined for antimicrobial activity against several human-, aqua-, and plant-pathogenic microbes. Compounds 5, 7, and 11 showed activity against the aquatic pathogen *Pseudomonas aeruginosa*, with MIC values of 8.0, 1.0, and 0.5 µg/mL, respectively, which are comparable to that of the positive control, chloramphenicol (MIC = 0.5 µg/mL). Moreover, Compounds 5, 7, 8, and 11 exhibited activity against the human pathogen *Escherichia coli* with MIC values of 16.0, 4.0, 0.5, and 2.0 µg/mL, respectively, while the positive control chloramphenicol has an MIC value of 1.0 µg/mL. Compounds 2, 5, 7, 9, and 11 exhibited activity against the aquatic pathogen *Vibrio parahaemolyticus* with MIC values of 64.0, 8.0, 2.0, 16.0, and 0.5 µg/mL, respectively, while the positive control chloramphenicol has an MIC value of 0.5 µg/mL. Compounds 5, 7, and 11 exhibited activity against *V. alginolyticus* with MIC values of 4.0, 1.0, and 0.5 µg/mL, respectively, while the positive control chloramphenicol has an MIC value of 0.5 µg/mL.

These data indicated that Compound 5, with a prenyl group at C-20, has stronger antimicrobial activity than does Compound 6, where the prenyl substituent is at C-21, while the results from Compounds 8, 9, and 10 showed that the double bond at C-11 and the hydroxyl substitute at C-10 significantly influenced the activity. Moreover, acetylation of OH at C-7 decreased the antibacterial activity (3 vs. 7).

3. Experimental Section

3.1. General

Melting points were determined with an SGW X-4 micro-melting-point apparatus. Optical rotations were measured on an Optical Activity AA-55 polarimeter (Optical Activity Ltd., Cambridgeshire, UK). UV spectra were measured on a Lengguang Gold S54 spectrophotometer (Shanghai Lengguang Technology Co. Ltd., Shanghai, China). ECD spectra were acquired on a JASCO J-715 spectropolarimeter (JASCO, Tokyo, Japan). The ¹H, ¹³C, and 2D NMR spectra were acquired using Bruker Avance 500 spectrometers (Bruker Biospin Group, Karlsruhe, Germany). Mass spectra were determined on a VG Autospec 3000 (VG Instruments, London, UK) or an API QSTAR Pulsar 1 mass spectrometer (Applied Biosystems, Foster, Waltham, MA, USA). Analytical HPLC and semi-preparative 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) (Dionex, Sunnyvale, CA, USA). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Factory, Qingdao, China), Lobar LiChroprep RP-18 (40–60 µm, Merck, Darmstadt, Germany), and Sephadex LH-20 (18–110 µm, Merck).

3.2. Fungal Material

The fungus *Penicillium* sp. AS-79 was isolated from the fresh tissue of the sea anemone *Haliplanella luciae*, which was collected from the Qingdao coastline in December 2014. The fungus was identified by sequence analysis of the ITS [21] and beta-tubulin [22] region of its rDNA as described previously. The resulting sequence data obtained from the fungal strain have been deposited in GenBank (with accession No. KY322516). A BLAST search result indicated that the ITS sequence was most similar (99%) to the sequence of *Penicillium janthinellum* (compared to KM268705.1), but the beta-tubulin sequence was most similar (95%) to *P. vasconiae* (compared to KT887805.1). Therefore, it is hard to identify the strain to a species level. The strain is preserved at the key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences.

3.3. Fermentation

The fermentation was carried out statically on a rice solid medium (each flask contained 70 g of rice (Cofco, Beijing, China), 0.1 g of corn flour (Macklin, Shanghai, China), 0.3 g of peptone (Shuangxuan, Beijing, China), 0.1 g of sodium glutamate (Lianhua, Henan, China), and 100 mL of naturally sourced

and filtered seawater, which was obtained from the Huiquan Gulf of the Yellow Sea near the campus of the authors' institution, pH 6.5–7.0) in 1 L Erlenmeyer flasks for 30 days at room temperature.

3.4. Extraction and Isolation

The whole fermented broth (66 flasks) was extracted exhaustively with EtOAc, yielding 72.5 g of crude extract, which was subjected to silica gel vacuum liquid chromatography (VLC) eluting with mixed solvents of increasing polarity (petroleum ether (PE) to MeOH) to yield nine fractions (Fraction 1 to Fraction 9). Fraction 3 (5.1 g) was further purified by reverse-phase column chromatography (CC) over Lobar LiChroprep RP-18 with a MeOH–H₂O gradient (from 20:80 to 100:0) to afford two subfractions (Fraction 3.1 and Fraction 3.2). Fraction 3.2 was further purified by prep. TLC (plate: 20 × 20 cm, developing solvents: PE/acetone, 10:1), then by CC on Sephadex LH-20 (MeOH), and finally by prep. TLC (plate: 20 × 20 cm, developing solvents: CH₂Cl₂/MeOH, 40:1) to obtain Compounds **3** (71.8 mg) and **11** (12.4 mg). Fraction 4 (2.0 g) was further purified by CC on silica gel (eluted with CH₂Cl₂-MeOH, 200:1 to 60:1), then on Sephadex LH-20 (MeOH) and finally on silica gel (eluted with CH₂Cl₂) to obtain Compound **6** (11.8 mg). Fraction 5 (5.9 g) was further purified by CC over Lobar LiChroprep RP-18 with a MeOH–H₂O gradient (from 20:80 to 100:0) to afford eight subfractions (Fraction 5.1–Fraction 5.8). Fraction 5.4 and Fraction 5.5 was further purified by CC on silica gel (eluted with CH₂Cl₂-MeOH, 100:1 to 60:1). Then Fraction 5.4 was purified by prep. TLC (plate: 20 × 20 cm, developing solvents: CH₂Cl₂/MeOH, 40:1) to obtain Compound **1** (12.3 mg). Fraction 5.5 (3.5 g) was further purified by CC on Sephadex LH-20 (MeOH) to obtain Compound **2** (5.2 mg). Fraction 5.7 (48.9 mg) was further purified by CC on silica gel (eluted with CH₂Cl₂-MeOH, 200:1 to 100:1) to obtain Compounds **4** (5.8 mg) and **8** (8.9 mg). Fraction 5.8 (139 mg) was further purified by CC on silica gel (eluted with CH₂Cl₂-MeOH, 200:1 to 100:1) and by prep. TLC (plate: 20 × 20 cm, developing solvents: CH₂Cl₂/MeOH, 80:1) to obtain Compounds **5** (10.0 mg) and **7** (9.7 mg). Fraction 6 (5.8 g) was further purified by CC over Lobar LiChroprep RP-18 with a MeOH–H₂O gradient (from 20:80 to 100:0) to afford five subfractions (Fraction 6.1–Fraction 6.5). Fraction 6.4 (100.0 mg) was further purified by CC on silica gel (eluted with PE-EtOAc, 10:1 to 8:1) to obtain Compound **9** (4.9 mg). Fraction 6.5 was further purified by prep. HPLC (MeOH–H₂O, 85:15) to obtain Compound **10** (11.2 mg, *t*_R 13.0 min).

22-Hydroxylshearinine F (**1**): colorless solid; $[\alpha]_D^{20}$: +50 (*c* 0.1, MeOH); UV (MeOH) λ_{\max} (log ϵ) 215 (4.39), 251 (4.29), 317 (4.02) nm; ECD λ_{\max} ($\Delta\epsilon$) 237.5 (−12.18), 272 (+3.54) nm; ¹H and ¹³C NMR data, see Table 1; ESIMS *m/z* 600 [M + H]⁺; HRESIMS *m/z* 600.3330 [M + H]⁺ (calcd. for C₃₇H₄₆NO₆, 600.3320).

6-Hydroxylpaspalinine (**2**): yellowish solid; $[\alpha]_D^{20}$: +30.8 (*c* 0.13, MeOH); UV (MeOH) λ_{\max} (log ϵ) 221 (3.51), 232 (3.49) nm; ECD λ_{\max} ($\Delta\epsilon$) 250 (−9.79), 354.5 (+30.62) nm; ¹H and ¹³C NMR data, see Table 1; ESIMS *m/z* 434 [M + H]⁺; HRESIMS *m/z* 434.2332 [M + H]⁺ (calcd. for C₂₇H₃₂NO₄, 434.2326).

7-O-Acetylemindole SB (**3**): colorless crystals; m.p. 192–194 °C; $[\alpha]_D^{20}$: +45.5 (*c* 0.11, MeOH); UV (MeOH) λ_{\max} (log ϵ) 229 (3.80), 281 (3.17) nm; ECD λ_{\max} ($\Delta\epsilon$) 250 (+6.50), 295 (−2.79) nm; ¹H and ¹³C NMR data, see Table 1; ESIMS *m/z* 448 [M + H]⁺; HRESIMS *m/z* 448.3207 [M + H]⁺ (calcd. for C₃₀H₄₂NO₂, 448.3210).

3.5. X-ray Crystallographic Analysis of Compound **3**

All crystallographic data were collected on an Agilent Xcalibur Eos Gemini CCD plate diffractometer (Agilent Technologies, Santa Clara, CA, USA), using graphite monochromatized Cu K α radiation ($\lambda = 1.54178$ Å) for **3** [23]. The data were corrected for absorption by using the program SADABS [24]. The structures were solved by direct methods with the SHELXTL software package (Sheldrick G.M., University of Göttingen, Germany) [25]. 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 [26].

Crystal data for compound 3: C₃₀H₄₁NO₂, F.W. = 447.64, orthorhombic space group, P2(1)2(1)2(1), unit cell dimensions $a = 6.8164(4) \text{ \AA}$, $b = 7.6058(6) \text{ \AA}$, $c = 50.959(3) \text{ \AA}$, $V = 2641.9(3) \text{ \AA}^3$, $\alpha = \beta = \gamma = 90^\circ$, $Z = 4$, $d_{\text{calcd}} = 1.125 \text{ mg/m}^3$, crystal dimensions $0.40 \times 0.33 \times 0.14 \text{ mm}$, $\mu = 0.531 \text{ mm}^{-1}$, $F(000) = 976$. The 4587 measurements yielded 306 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave $R_1 = 0.0641$ and $wR_2 = 0.1236 [I > 2\sigma(I)]$. The final refinement Flack parameter is 0.0(5).

3.6. Antimicrobial Assay

Antimicrobial assay against human- and aqua-pathogenic microbes *Edwardsiella tarda*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *V. harveyi*, *V. parahaemolyticus*, and plant pathogenic fungi *Alternaria alternata*, *A. brassicae*, *Colletotrichum gloeosporioides*, *Fusarium graminearum*, *F. oxysporum*, *Gaeumannomyces graminis*, *Phytophthora nicotiana*, *Physalospora piricola*, and *Valsa mali* was carried out using the well diffusion method [27]. Chloromycetin was used as a positive control for the bacteria, while amphotericin B was used as a positive control for the fungi.

4. Conclusions

Three new indolediterpenoid derivatives (1–3), along with eight known analogs (4–11), were isolated from the sea anemone-derived fungus *Penicillium* sp. AS-79. The structures and relative configurations were determined via the interpretation of NMR data, and the absolute configurations of all compounds were determined via ECD comparison, while the structure of Compound 3 was confirmed by single-crystal X-ray diffraction analysis. Several compounds exhibited activity against some of the tested microbial strains.

Supplementary Materials: The following are available online at www.mdpi.com/1660-3397/15/5/137/s1. 1D and 2D NMR spectra of Compounds 1–3 as well as crystal packing of Compound 3.

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Author Contributions: X.-Y.H. performed the experiments of isolation, structure elucidation, and antimicrobial evaluation, and prepared the manuscript; L.-H.M. contributed to part of the structure determination; X.L. performed the ECD calculations, S.-Q.Y. isolated the fungus from sea-anemone; X.-M.L. performed the 1D and 2D NMR experiments, B.-G.W. supervised the research work and revised the manuscript.

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References

1. Sun, K.L.; Li, Y.; Guo, L.; Wang, Y.; Liu, P.P.; Zhu, W.M. Indolediterpenoids and isocoumarin from the fungus, *Aspergillus flavus*, isolated from the prawn, *Penaeus vannamei*. *Mar. Drugs* **2014**, *12*, 3970–3981. [[CrossRef](#)] [[PubMed](#)]
2. Saikia, S.; Nicholson, M.J.; Young, C.; Parker, E.J.; Scott, B. The genetic basis for indole-diterpene chemical diversity in filamentous fungi. *Mycol. Res.* **2008**, *112*, 184–199. [[CrossRef](#)] [[PubMed](#)]
3. Byrne, K.M.; Smith, S.K.; Ondeyka, J.G. Biosynthesis of nodulisporic acid A: Precursor studies. *J. Am. Chem. Soc.* **2002**, *124*, 7055–7060. [[CrossRef](#)] [[PubMed](#)]
4. Qiao, M.F.; Ji, N.Y.; Liu, X.H.; Li, K.; Zhu, Q.M.; Xue, Q.Z. Indoloditerpenes from an algicolous isolate of *Aspergillus oryzae*. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5677–5680. [[CrossRef](#)] [[PubMed](#)]
5. Ding, L.; Maier, A.; Fiebig, H.H.; Lin, W.H.; Hertweck, C. A family of multicyclic indolosesquiterpenes from a bacterial endophyte. *Org. Biomol. Chem.* **2011**, *9*, 4029–4031. [[CrossRef](#)] [[PubMed](#)]
6. Hensens, O.D.; Ondeyka, J.G.; Dombrowski, A.W.; Ostlind, D.A.; Zink, D.L. Isolation and structure of nodulisporic acid A₁ and A₂, novel insecticides from a *Nodulisporium* sp. *Tetrahedron Lett.* **1999**, *40*, 5455–5458. [[CrossRef](#)]

7. Knaus, H.G.; McManus, O.B.; Lee, S.H.; Schmalhofer, W.A.; Garcia-Calvo, M.; Helms, L.M.H.; Sanchez, M.; Giangiacomo, K.; Reuben, J.P.; Smith, A.B., III; et al. Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. *Biochemistry* **1994**, *33*, 5819–5828. [[CrossRef](#)] [[PubMed](#)]
8. Li, C.; Gloer, J.B.; Wicklow, D.T.; Dowd, P.F. Thiersinines A and B: Novel antiinsectan indole diterpenoids from a new fungicolous *Penicillium* species (NRRL 28147). *Org. Lett.* **2002**, *4*, 3095–3098. [[CrossRef](#)] [[PubMed](#)]
9. Ondeyka, J.G.; Helms, G.L.; Hensens, O.D.; Goetz, M.A.; Zink, D.L.; Tsipouras, A.; Shoop, W.L.; Slayton, L.; Dombrowski, A.W.; Polishook, J.D.; et al. Nodulisporic acid A, a novel and potent insecticide from a *Nodulisporium* sp. isolation, structure determination, and chemical transformations. *J. Am. Chem. Soc.* **1997**, *119*, 8809–8816. [[CrossRef](#)]
10. Du, F.Y.; Zhang, P.; Li, X.M.; Li, C.C.; Cui, C.M.; Wang, B.G. Cyclohexadepsipeptides of the isaridin class from the marine-derived fungus *Beauveria felina* EN-135. *J. Nat. Prod.* **2014**, *77*, 1164–1169. [[CrossRef](#)] [[PubMed](#)]
11. Meng, L.H.; Wang, C.Y.; Mándi, A.; Li, X.M.; Hu, X.Y.; Kassack, U.M.; Kurtán, T.; Wang, B.G. Three diketopiperazine alkaloids with spirocyclic skeletons and one bithiodiketopiperazine derivative from the mangrove-derived endophytic fungus *Penicillium brocae* MA-231. *Org. Lett.* **2016**, *18*, 5304–5307. [[CrossRef](#)] [[PubMed](#)]
12. Li, H.L.; Li, X.M.; Liu, H.; Meng, L.H.; Wang, B.G. Two new diphenylketones and a new xanthone from *Talaromyces islandicus* EN-501, an endophytic fungus derived from the marine red alga *Laurencia okamurai*. *Mar. Drugs* **2016**, *14*, 223. [[CrossRef](#)] [[PubMed](#)]
13. Xu, M.J.; Gessner, G.; Groth, I.; Lange, C.; Christner, A.; Bruhn, T.; Deng, Z.W.; Li, X.; Heinemann, S.H.; Grabley, S.; et al. Shearinines D–K, new indole triterpenoids from an endophytic *Penicillium* sp. (strain HKI0459) with blocking activity on large-conductance calcium-activated potassium channels. *Tetrahedron* **2007**, *63*, 435–444. [[CrossRef](#)]
14. Sun, H.; Gao, S.S.; Li, X.M.; Li, C.S.; Wang, B.G. Chemical constituents of marine mangrove-derived endophytic fungus *Alternaria tenuissima* EN-192. *Chin. J. Oceanol. Limnol.* **2013**, *31*, 464–470. [[CrossRef](#)]
15. Harms, H.; Rempel, V.; Kehraus, S.; Kaiser, M.; Hufendiek, P.; Müller, C.E.; König, G.M. Indoloditerpenes from a marine-derived fungal strain of *Dichotomomyces cejpaii* with antagonistic activity at GPR18 and cannabinoid receptor. *J. Nat. Prod.* **2014**, *77*, 673–677. [[CrossRef](#)] [[PubMed](#)]
16. Dorner, W.J.; Cole, J.R.; Cox, H.R.; Cunfer, M.B. Paspalitrein C, a new metabolite from sclerotia of *Claviceps paspali*. *J. Agric. Food Chem.* **1984**, *32*, 1069–1071. [[CrossRef](#)]
17. Springer, P.J.; Clardy, J.; Wells, M.J.; Cole, J.R.; Kirksey, W. The structure of paxilline, a tremorgenic metabolite of *Penicillium paxilli* bainier. *Tetrahedron Lett.* **1975**, *16*, 2531–2534. [[CrossRef](#)]
18. Fan, Y.Q.; Wang, Y.; Liu, P.P.; Fu, P.; Zhu, T.H.; Wang, W.; Zhu, W.M. Indole-diterpenoids with anti-H1N1 activity from the aciduric fungus *Penicillium camemberti* OUCMDZ-1492. *J. Nat. Prod.* **2013**, *76*, 1328–1337. [[CrossRef](#)] [[PubMed](#)]
19. Hosoe, T.; Nozawa, K.; Udagawa, S.I.; Nakajima, S.; Kawai, K.I. Structures of new indoloditerpenes, possible biosynthetic precursors of the tremorgenic mycotoxins, penitremes, from *Penicillium crustosum*. *Chem. Pharm. Bull.* **1990**, *38*, 3473–3475. [[CrossRef](#)]
20. Tepaske, R.M.; Gloer, B.J.; Wicklow, T.D.; Dowd, F.P. Three new aflavinines from sclerotia of *Aspergillus tubingensis*. *Tetrahedron* **1989**, *45*, 4961–4968. [[CrossRef](#)]
21. Wang, S.; Li, X.M.; Teuscher, F.; Li, D.L.; Diesel, A.; Ebel, R.; Proksch, P.; Wang, B.G. Chaetopyranin, a benzaldehyde derivative, and other related metabolites from *Chaetomium globosum*, an endophytic fungus derived from the marine red alga *Polysiphonia urceolata*. *J. Nat. Prod.* **2006**, *69*, 1622–1625. [[CrossRef](#)] [[PubMed](#)]
22. Visagie, C.M.; Houbraken, J.; Frisvad, J.C.; Hong, S.-B.; Klaassen, C.H.W.; Perrone, G.; Seifert, K.A.; Varga, J.; Yaguchi, T.; Samson, R.A. Identification and nomenclature of the genus *Penicillium*. *Stud. Mycol.* **2014**, *78*, 343–371. [[CrossRef](#)] [[PubMed](#)]
23. Crystallographic Data of Compound 3 Have Been Deposited in the Cambridge Crystallographic Data Centre (CCDC 1529887). Available online: http://www.ccdc.cam.ac.uk/data_request/cif (accessed on 11 May 2017).
24. Sheldrick, G.M. *SADABS, Software for Empirical Absorption Correction*; University of Göttingen: Göttingen, Germany, 1996.

25. Sheldrick, G.M. *SHELXTL, Structure Determination Software Programs*; Bruker Analytical X-ray System Inc.: Madison, WI, USA, 1997.
26. Sheldrick, G.M. *SHELXL-97 and SHELXS-97, Program for X-ray Crystal Structure Solution and Refinement*; University of Göttingen: Göttingen, Germany, 1997.
27. Al-Burtamani, S.K.S.; Fatope, M.O.; Marwah, R.G.; Onifade, A.K.; Al-Saidi, S.H. Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman. *J. Ethnopharmacol.* **2005**, *96*, 107–112. [[CrossRef](#)] [[PubMed](#)]



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