

A New Sesquiterpene from the Fungus *Penicillium* sp. LPFH-hzw-zw1

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Abstract: An investigation of the secondary metabolites of the sea sediment-derived *Penicillium* sp. LPFH-ZW-1 resulted in the identification of a new compound, named penicipeneacid (**1**), along with seven known compounds. The structures of these compounds were established by extensive analysis of the 1D and 2D NMR data, combined with HRESIMS data. The absolute configuration of **1** was determined by comparing the experimental and predicted ECD spectra. The bicyclo[4.1.0]heptane nucleus in compounds **1** and **2** was rarely found in nature. The known compounds were identified to be sesquicaranoic acid B (**2**), 9-cycloneren-3,7,11-triol (**3**), fumiquinazoline J (**4**), versiquinazoline H (**5**), penipurdin A (**6**), questinol (**7**), and 2-hydroxy-4-ethoxybenzoic acid (**8**). Compounds **4** and **7** exhibited moderate cytotoxicity against MCF-7 and K562 tumor cell lines, with IC₅₀ values ranging from 12.6 to 37.1 μM.

Keywords: marine fungus; *Penicillium*; sesquiterpene; penicipeneacid © 2024 ACG Publications. All rights reserved.

1. Introduction

In recent years, marine fungi have become the focus of research on marine biological resources, particularly those found in sea sediment, mangroves, sponges, and corals. Numerous compounds with unique structures and impressive activities have been discovered from these sources [1, 2]. Among them, there are a large number of *Penicillium* strains, from which terpenoids [3], polyketides [4], and alkaloids [5] have been identified. The genus *Penicillium* has been recognized to be a prolific source of new metabolites with fascinating structures or significant activities. Here are some excellent examples. Meroantarctines A–C, meroterpenoids with three unique skeletons, were isolated from *P. antarcticum* [6]. Culture of a soft coral-derived *Penicillium* strain yielded the decahydro-fluorene alkaloids pyrrospirones K–Q. Among these, pyrrospirones K and L had a 6/5/6/8/5/6/13 polycyclic skeleton while pyrrospirones M and N possessed a 6/5/6/5/6/13 polycyclic skeleton [7]. Penicillipyron B, a novel meroterpenoid from a sediment-derived *Penicillium* strain, exhibited significant induction of quinone reductase in murine hepatoma cells [8]. An endophytic *Penicillium* sp. from mangroves was the source of a spiroax-4-ene-12-one derivative, which showed potent inhibitory effects towards the MG-63 cells and human osteosarcoma in nude mice following oral administration [9]. Chrysamides A–C are dimeric nitrophenyl *trans*-epoxyamides produced by *Penicillium chrysogenum* derived from deep-sea sediment, the former two have a unique centrosymmetric dimer skeleton, and chrysamide C suppresses the production of the pro-inflammatory cytokine interleukin-17 [10].

In our quest for new/bioactive metabolites from marine fungi, we isolated a new sesquiterpene, named penicipeneacid (**1**), along with seven known compounds (**2–8**) from the marine-derived strain

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Penicillium sp. LPFH-hzw-zw1 (Figure 1). Details describing the isolation, structure elucidation, and bioactivity evaluation of the metabolites are disclosed herein.

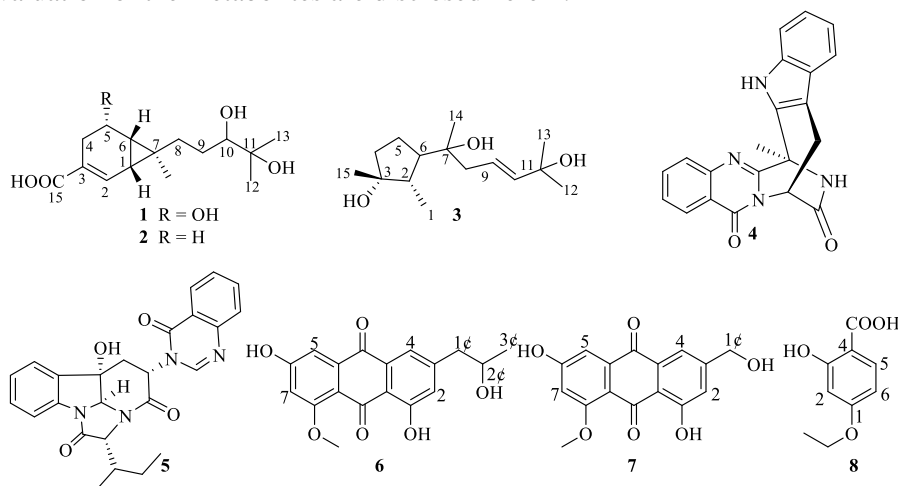


Figure 1. Compounds 1–8 from the marine fungus *Penicillium* sp. LPFH-hzw-zw1

2. Materials and Methods

2.1. General Experimental Procedures

The UV spectrum was tested using a Cary 300 spectrometer. The 1D (^1H NMR, ^{13}C NMR) and 2D (HSQC, HMBC, COSY, and NOESY) NMR data were recorded on a Bruker Avance-400FT NMR spectrometer. HRESIMS data was recorded on a Waters Xevo G2 Q-TOF spectrometer equipped with an ESI source. Semi-preparative high-performance liquid chromatography (HPLC) was conducted using a Shimadzu LC-6AD pump equipped with a UV detector. The separation was carried out on a YMC-Pack ODS-A column (AA12S05-2510WT).

2.2. Microorganism Material

The strain LPFH-hzw-zw1 was obtained from a sediment sample collected from Hangzhou Bay and was identified as *Penicillium* sp. based on comparisons of its internal transcribed spacer rDNA (ITS rDNA) gene sequences with that of a strain (OQ704017) in the GenBank (www.ncbi.nlm.nih.gov/genbank). The strain is preserved at the First People's Hospital of Linping District, Hangzhou, China.

2.3. Fermentation and Isolation

The strain was cultured on solid rice medium in 30 fernbach flasks (500 mL), each containing 70 g of rice and 90 mL of filter-sterilized seawater. The contents were left at room temperature (r.t.) for two hours and then autoclaved for 15 minutes. The fresh mycelia were propagated on PDA medium for 4 days and then inoculated into the fernbach flasks. The fermentation was conducted at r.t. for approximately 30 days. The fermented materials were extracted three times using the solvent EtOAc (10 L) to obtain the extraction solution, which was then concentrated under vacuum to yield the extract.

The extract (2.6 g) was separated using a silica gel column with a gradient (100:1 to 0:100) of petroleum ether/ethyl acetate to obtain four fractions (F1–F4).

Fraction F1 was split into five fractions using ODS silica gel CC with MeOH/H₂O (20:80 to 100:0) as the eluent, resulting in six subfractions F2a–F2f. Fraction F2f was further purified using an HPLC system equipped with a C-18 column and MeOH/H₂O (40:60, 2 mL/min) as the mobile phase, yielding **1** (2.7 mg) and **2** (10.6 mg).

Fraction F2 was separated on an ODS silica gel to obtain four subfractions F2a–F2d (30:70 to

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100:0). Subfraction F2b was then further purified by HPLC using a C-18 column with MeOH/H₂O (45:55, 2 mL/min) to yield compound **3** (11 mg). F2C underwent additional purification using HPLC with MeOH/H₂O (47:53, 2 mL/min) to yield compound **6** (2.2 mg).

Fraction F3 was chromatographed using an ODS silica gel to yield six subfractions F2a–F2f (30:70 to 100:0). Subfraction F2d was purified via HPLC with elution using MeOH/H₂O (50:50, 2 mL/min) to obtain compounds **6** and **7**. Subfraction F2f was isolated through HPLC using CH₃CN/H₂O (50:50, 2 mL/min) to yield compound **5** (7.8 mg).

Fraction F4 was subjected to an ODS silica gel using MeOH/H₂O (30:70 to 100:0) to yield five subfractions (F4a–F4e). F4c was purified by HPLC using a mixture of acetonitrile (ACN) and water (60:40, 2 mL/min) to yield compound **4** (3.0 mg).

Penicipeneacid (1): Colorless oil; $[\alpha]_{\text{D}}^{25} +142$ ($c = 0.1$, CH₃OH); UV (MeOH) λ_{max} 242 (4.67) nm; ECD ($c = 8.0 \times 10^{-4}$ M, MeOH) λ_{max} ($\Delta\epsilon$) 255 (+2.11), 207 (−2.95) nm; ¹H and ¹³C NMR data, Table 1; HRESIMS m/z 307.1503 [$M + \text{Na}$]⁺ (calcd for C₁₅H₂₄O₅Na⁺, 307.1516).

Fumiquinazoline J (4): ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.25 (s, 1H), 9.60 (s, 1H), 8.15 (dd, $J = 8.1$, 1.6 Hz, 1H), 7.66 (d, $J = 8.1$ Hz, 1H), 7.54 (t, $J = 8.0$ Hz, 1H), 7.41 (d, $J = 7.9$ Hz, 1H), 7.37 (d, $J = 8.2$ Hz, 1H), 7.12 (td, $J = 7.6$, 6.9, 1.2 Hz, 1H), 6.99 (t, $J = 7.5$ Hz, 1H), 5.70 (t, $J = 3.6$ Hz, 1H), 3.43 (dd, $J = 17.4$, 2.8 Hz, 1H), 3.23 (dd, $J = 17.4$, 4.6 Hz, 1H).

Versiquinazoline H (5): ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.30 (s, 1H), 8.18 (d, $J = 8.0$ Hz, 1H), 7.87 (t, $J = 7.7$ Hz, 1H), 7.71 (d, $J = 8.1$ Hz, 1H), 7.60 (d, $J = 7.6$ Hz, 1H), 7.56 (d, $J = 7.8$ Hz, 1H), 7.48 (d, $J = 7.7$ Hz, 1H), 7.44 (t, $J = 7.6$ Hz, 1H), 7.27 (t, $J = 7.3$ Hz, 1H), 5.81 (s, 1H), 4.84 (dd, $J = 8.3$, 5.4 Hz, 1H), 4.27 (d, $J = 9.4$ Hz, 1H), 3.05 (dd, $J = 13.8$, 8.7 Hz, 1H), 2.78 (dd, $J = 13.9$, 5.3 Hz, 1H), 2.31–2.14 (m, 1H), 1.84–1.64 (m, 1H), 1.28 (dq, $J = 17.6$, 9.9, 8.8 Hz, 2H), 1.12 (d, $J = 6.6$ Hz, 3H), 0.97 (t, $J = 7.3$ Hz, 3H).

2.4. Cytotoxicity Assay

The cytotoxicities of compounds **1–8** towards human tumor cells MCF-7 and K562 were tested using the MTT method. The cells were plated in 96-well plates for 24 hours and then exposed to the compounds (at a concentration of 50 μM) for 48 h. The IC₅₀ values for those with inhibition rate more than 50% were further determined. Experiments were repeated three times and conducted in triplicate.

3. Results and Discussion

3.1. Structure Elucidation

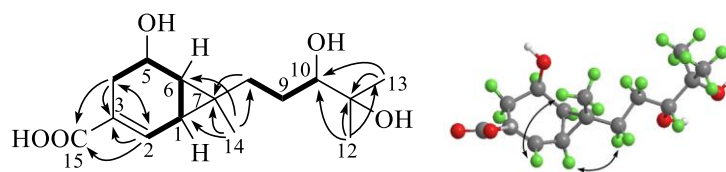
Compound **1** was obtained as a colorless oil. Its molecular formula was determined to be C₁₅H₂₄O₅ on the basis of the pseudomolecular ion peak at m/z 307.1503 (calcd for C₁₅H₂₄O₅Na⁺, 307.1516) in the HRESIMS spectrum, indicating four sites of unsaturation. The ¹H NMR spectrum displayed the resonances for three tertiary methyl groups, two of which were connected to an oxygenated carbon (δ_{H} 1.18, 1.15), an olefinic proton (δ_{H} 7.21), two oxygenated protons (δ_{H} 4.19, 3.29), and a series of alkyl protons. The 15 carbon resonances in the ¹³C NMR spectrum, combined with the HSQC spectrum, were classified into two olefinic carbons (δ_{C} 140.2 and 127.3), a carboxylic carbon (δ_{C} 170.5), three methyl carbons (δ_{C} 25.6, 24.8, 13.6), three methylene carbons (δ_{C} 42.0, 33.2, and 29.5), four sp³ methine carbons (δ_{C} 79.2, 66.6, 33.7, 28.4), and two sp³ non-protonated carbons including an oxygenated one (δ_{C} 73.9, 35.2). An α,β -unsaturated acid moiety was deduced based on the chemical shifts of the olefinic carbons. Two of the four degrees of unsaturation were explained by the α,β -unsaturated acid moiety, while the remaining two indicated that compound **1** was bicyclic.

Table 1. ^1H (400 Hz) and ^{13}C NMR (100 Hz) data of **1** and **2** (δ in ppm) in CD_3OD .

No.	1		2		3
	δ_{H} , mult. (<i>J</i> in Hz)	δ_{C}	δ_{H} , mult. (<i>J</i> in Hz)	δ_{C}	δ_{C}
1	1.61, dd (8.0, 5.7)	28.4, CH	1.32, m	24.2, CH	15.2, CH_3
2	7.21, ddd (5.7, 2.8, 2.8)	140.2, CH	7.24, ddd (5.5, 2.7, 2.7)	140.9, CH	43.5, CH
3		127.3, C		128.6, C	79.1, C
4	2.86, dd (17.8, 8.3), 1.74, m	33.2, CH_2	1.89, m; 2.36, m	22.7, CH_2	40.4, CH_2
5	4.19, ddd (14.0, 8.3, 5.4)	66.6, CH	1.80, m; 1.90, m	18.2, CH	23.8, CH_2
6	1.51, dd (8.0, 5.4)	33.7, CH	1.25, m	26.3, CH	53.7, CH
7		35.2, C		34.0, C	73.4, C
8	1.74, m; 1.26, m	42.0, CH_2	1.71, m; 1.25, m	41.5, CH_2	43.9, CH_2
9	1.82, m; 1.39, m	29.5, CH_2	1.76, m; 1.37, m	29.3, CH_2	121.9, CH
10	3.29, dd (9.6, 1.4)	79.2, CH	3.22, m	79.4, CH	141.3, CH
11		73.9, C		73.9, C	69.0, C
12	1.15, s	24.8, CH_3	1.15, s	24.9, CH_3	26.3, CH_3
13	1.18, s	25.6, CH_3	1.18, s	25.7, CH_3	30.1, CH_3
14	1.0, s	13.6, CH_3	0.92, s	13.4, CH_3	30.1, CH_3
15		170.5, C		170.9, C	30.1, CH_3

The structure of compound **1**, which features a bicyclic nucleus and a side chain, was determined by extensive analysis of the 2D NMR data (Figure 2). The COSY relationship [H_2 -8 (δ_{H} 1.74, 1.26)/ H_2 -9 (δ_{H} 1.82, 1.39)/ H_2 -10 (δ_{H} 3.29) and H_2 -4 (δ_{H} 2.86, 1.74)/ H_2 -5 (δ_{H} 4.19)/ H_2 -6 (δ_{H} 1.51)/ H_2 -1 (δ_{H} 1.61)/ H_2 -2 (δ_{H} 7.21)] revealed two spin systems. The HMBC correlations from H_2 -4 to C-2, C-3, C-4 and from H_3 -14 to C-1, C-6, and C-7 established the bicyclo[4.1.0]heptane nucleus. Additional HMBC correlations from H_3 -12 (δ_{H} 1.18) and H_3 -13 (δ_{H} 1.15) to C-10 (δ_{C} 79.2) and C-11 (δ_{C} 73.9) allowed the connection of a propan-2-ol unit to the spin system C-8/C-9/C-10 at C-10, constructing an eight-carbon side chain. The side chain was linked to C-7 by the HMBC correlations from H_2 -8 (δ_{H} 1.26) to C-7 (δ_{C} 35.2). The three oxygenated carbons were each linked to a hydroxy group, as deduced by the molecular formula. Hence, the gross structure of compound **1** was determined.

The relative configuration of compound **1** was determined using a NOESY experiment and the coupling constants. The NOESY interactions between H_3 -14 (δ_{H} 1.90) and H_2 (δ_{H} 7.21) and from H_2 -8a (δ_{H} 1.26) to H_2 -1 (δ_{H} 1.61), combined with the coupling constant between H_2 -1 and H_2 -6 (8.0 Hz) revealed that H_2 -1 and H_2 -6 were in the same orientation, while H_3 -14 was in the opposite orientation.

**Figure 2.** Key ^1H - ^1H COSY (—), HMBC (↷), and NOESY correlations (↶) of **1**

The absolute configuration of C-1, C-5, and C-6 was determined to be *R*, *S*, *S*, respectively, by comparing their ECD spectrum with the calculated ECD spectra of the simplified structures **1a** and its enantiomer **1b**, which were obtained by removing the flexible chain to create a rigid scaffold. Conformational analysis of **1b** was conducted using the MMFF94S force field with an energy cutoff of 3 kcal/mol to give three lowest energy conformers. These conformers were subsequently optimized using Density Functional Theory (DFT) at the B3LYP/6-31+G(d,p) level in MeOH. The ECD data of the optimized conformers were calculated at the B3LYP/6-31+G(d,p) level in methanol using the SMD model and were averaged according to their Boltzmann distribution (37.75%, 37.75%, 24.5%). The ECD spectrum of **1a** was obtained by inverting that of **1b**. As a result, the calculated ECD curve

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of **1a** exhibited a positive Cotton effect at 247 nm and a negative Cotton effect at 220 nm, similar to the experimental ECD spectrum of **1**. The absolute configuration of C-7 in compound **1** was assigned as *S* based on the relative configuration. The structure of compound **1** was thus established as depicted and was given the name penicipeneacid.

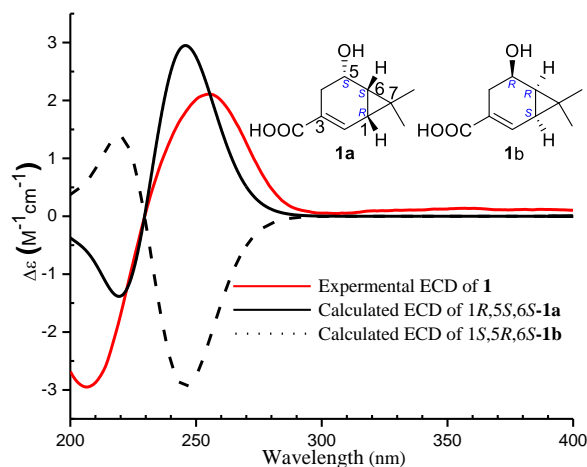


Figure 3. Experimental ECD spectrum of **1** and Calculated ECD spectra of the model molecule **1a** and **1b**

Table 2. ^1H NMR Data of **6-8** in $\text{DMSO-}d_6$ (400 MHz, δ in ppm, J in Hz)

No.	6	7	8
	δ_{H}	δ_{H}	δ_{H}
1			
2	7.15, d (1.2)	7.18, d (1.2)	6.29, s
4	7.49, d (1.2)	7.58, d (1.2)	
5	7.19, d (1.9)	7.21, d (1.9)	7.64, d (8.7)
6	6.86, d (1.9)	6.79, d (1.9)	6.33, d (8.7)
1'	2.73, dd (13.3, 5.2)	4.58, s	4.31, t (7.0)
2'	3.89, m		1.31, d (7.0)
3'	1.10, d (6.1)		
OCH_3	3.90, s	3.90, s	

Moreover, the remaining compounds were elucidated as sesquiterpene B (**2**) [11], 9-cycloneren-3,7,11-triol (**3**) [12], fumiquinazoline J (**4**) [13], versiquinazoline H (**5**) [14], penipurdin A (**6**) [15], questinol (**7**) [16], and 2-hydroxy-4-ethoxybenzoic acid (**8**) [17] based on comparisons of their NMR data with the reported data for compounds with identical gross structures in the literature. The ^1H NMR data of compounds **6-8** are listed in table 2.

3.2 Bioassay Study

All the compounds were tested for their cytotoxic effects against MCF-7 and K562 tumor cell lines at an initial concentrations of 50 μM . Only compounds **4** and **7** demonstrated weak inhibitory effects against the two tumor cells with inhibition rate more than 50%. Compounds **4** and **7** exhibited IC_{50} values of 12.6 and 37.1 μM against MCF-7 cells (IC_{50} of doxorubicin hydrochloride: 1.3 μM), respectively. While their IC_{50} values towards K562 cells were 17.2 and 25.9 μM (IC_{50} of 5-fluorouracil: 29.1 μM), respectively.

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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