

# Penicisepene, A New Sesquiterpenoid from the Fungus *Penicillium* sp. LPFH-Q3

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**Abstract:** The secondary metabolites of the strain *Penicillium* sp. LPFH-Q3 isolated from the sea sediment were investigated. A new eremophilane sesquiterpene, named penicisepene (**1**), along with seven known compounds (**2–8**) was obtained. The structures of the metabolites were elucidated based on spectroscopic analysis including <sup>1</sup>H NMR, <sup>13</sup>C NMR, <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, HMBC, NOESY, and the MS data. The absolute configuration of **1** was established via comparison of the experimental and density functional theory predicted ECD data. Compound **1**, an eremophilane sesquiterpene containing a hexenic acid, was scarcely found in nature. Besides, the NMR data of **3** and **4** in methanol-*d*<sub>4</sub> were reported for the first time. Biological evaluation showed that compounds **1**, **3**, and **4** showed inhibitory activity comparable to the positive control quercetin (16.7 μM) on NO production in LPS-activated RAW 264.7 macrophages with IC<sub>50</sub> values of 13.2, 10.6, 17.9 μM, respectively.

**Keywords:** *Penicillium* sp.; marine fungus; sesquiterpene; NO production. © 2023 ACG Publications. All rights reserved.

## 1. Introduction

Filamentous fungal strains such as species belonging to *Penicillium* or *Aspergillus* are important source of structurally distinctive molecules with promising pharmaceutical potential. Since the miraculous medicine penicillin G discovered from *Penicillium notatum* in 1928, species belonging to the genus have gained great attraction with reference to their metabolites and proven to be productive sources of molecules possessing pharmacological activity. The discovery of compactin with potent cholesterol-lowering from *P. citrinum* and the immunosuppressant mycophenolic acid from *P. brevicompactum* further proved that this genus is a potential source bioactive compounds.

In recent years, the chemistry study of *Penicillium* species led to the discovery of novel decahydrofluorene-class alkaloids (pyrrospirones K–Q) [1], unique hybrid diterpenic meroterpenoid (pyrandecarurins and pileotins) [2], seco and nor-seco isodihilarane-type meroterpenoids [3], pyridone alkaloids (penicipyridones) with anti-inflammatory activity [4], unusual indole-diterpenoid derivatives (oxalierpenes) with antiviral activity [5], bisafuranol from (-)- $\alpha$ -isabolol [6] and a rare lactam-type eremophilane [7].

In our search for new bioactive metabolites from marine fungi [8], a new eremophilane sesquiterpene (**1**), named penicisepene, together with seven known compounds (**2–8**) were isolated from the marine-derived strain *Penicillium* sp. LPFH-Q3 (Figure 1). Details describing the isolation, structure elucidation, and biological evaluation of the metabolites are disclosed, herein.

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## 2. Materials and Methods

### 2.1. General Experimental Procedures

UV spectrum was tested on a Cary 300 spectrometer. The  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, HSQC, HMBC, COSY, and NOESY spectra were recorded on a Bruker Avance-400FT NMR spectrometer. HRESIMS data was achieved on a Waters Xevo G2 Q-TOF spectrometer equipped with an ESI source. Semi-preparative HPLC was performed on a Shimadzu LC-6AD pump with a UV detector, and a YMC-Pack ODS-A column (AA12S05-2510WT) was used for separation.

### 2.2. Microorganism Material

The fungal strain LPFH-Q3 was isolated from a sediment sample collected from the Hangzhou Bay and was identified to be *Penicillium* sp. according to its morphology and internal transcribed spacer rDNA (ITS rDNA) gene sequences (OQ704017). The strain is preserved at the First People's Hospital of Linping District of Hangzhou, Hangzhou, China.

### 2.3. Fermentation and Isolation

The fermentation was performed in 20 fernbach flasks (500 mL), 80 g of rice and 90 mL of filter-sterilized seawater were added. The contents were autoclaved in a steam sterilizer for 15 min at 121.3 °C. The fresh mycelia of LPFH-Q3 were grown on PDA medium at room temperature (r.t.) for 3 days and were then transferred into the flasks. Fermentation was carried out at r.t. for approximately 30 days. The solid cultures were extracted three times with 4 L of EtOAc to give the solution, which was concentrated to afford an extract.

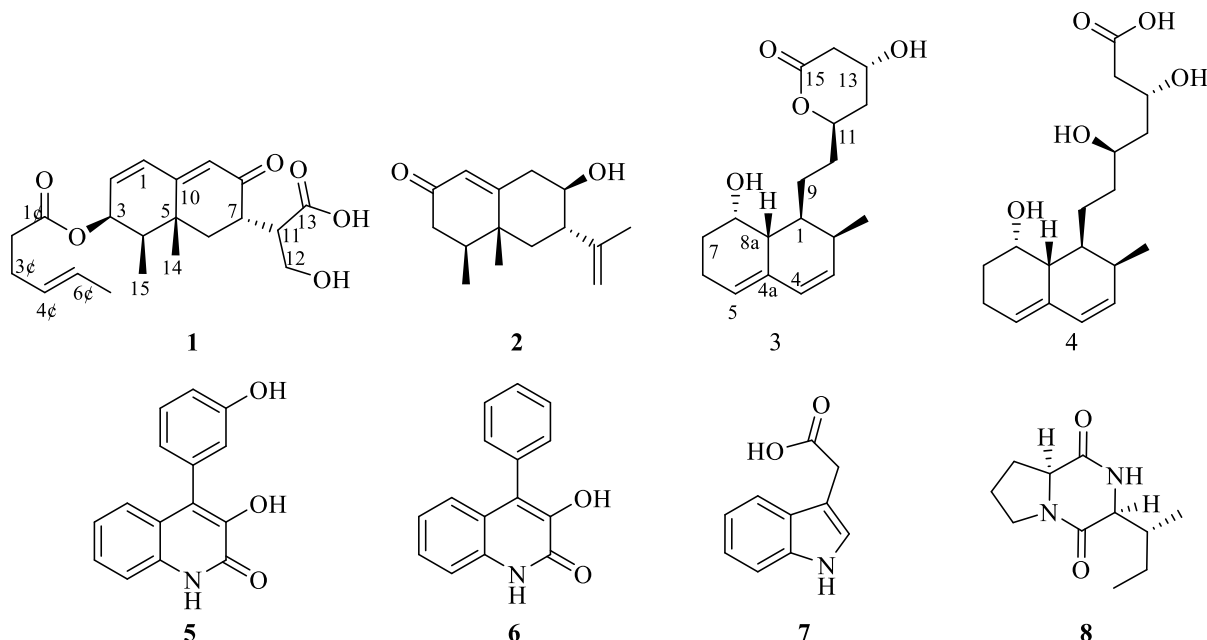
The extract (3.2 g) was split by middle chromatogram isolated gel (MCI) using MeOH/H<sub>2</sub>O (20:80→100:0) to give eight fractions (F1–F7). Fraction F2 was separated by ODS silica gel CC with MeOH/H<sub>2</sub>O (20:80 to 100:0) as eluent to obtain five subfractions F2a–F2e, Fraction F2b was separated over a HPLC column eluted with MeOH/H<sub>2</sub>O (40:60) to give **7** (3.6 mg). Fraction F2e was subject to further purification by HPLC eluted with MeOH/H<sub>2</sub>O (55:45, 3 mL/min) to give **4** (3.2 mg). Fraction F3 was subject to an ODS silica gel CC using MeOH/H<sub>2</sub>O (40:60→100:0) as eluent to afford four subfractions F3a–F3d, fraction F3a was separated by HPLC using ACN/H<sub>2</sub>O (45:55, 3 mL/min, C18 column) to obtain **5** (3.2 mg), fraction F3c was separated on HPLC using ACN/H<sub>2</sub>O (55:45, 3 mL/min) to obtain **6** (3.2 mg). Fraction F4 was split on an ODS silica gel CC to give five subfractions F4a–F4f, fraction F4c was separated by HPLC equipped with a semi-preparative YMC-pack ODS-A column (*S*-5 μm, 12 nm, 250 × 12 mm) using ACN/H<sub>2</sub>O (55:45, 3 mL/min, C18 column) to obtain **2** (16.0 mg) and **8** (5.0 mg), fraction F4e was separated by HPLC using ACN/H<sub>2</sub>O (60:40, 3 ml/min) to give **3**. F6 was subject to a silica gel using petroleum ether/ethyl acetate (10:1 to 2:1) to afford subfractions F6a– F6e, fraction 6e was purified on HPLC by MeOH/H<sub>2</sub>O (85:15) to give **1** (2.7 mg).

*Penicisepene* (**1**):  $[\alpha]_D^{25} +178$  (c 0.1, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 274 (4.17); ECD (c  $3.1 \times 10^{-4}$  M, MeOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 269 (+9.69), 347 (+2.32); HRESIMS  $m/z$  377.1956  $[\text{M} + \text{H}]^+$  (calcd. for  $\text{C}_{21}\text{H}_{29}\text{O}_6^+$ , 377.1959). HRESIMS  $m/z$  399.1774  $[\text{M} + \text{Na}]^+$  (calcd. for  $\text{C}_{21}\text{H}_{28}\text{O}_6\text{Na}^+$ , 399.1778).

### 2.4. Determination of NO Production.

The RAW 264.7 mouse macrophage cell line was routinely cultured in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, with penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37°C in a humidified, 5% CO<sub>2</sub> incubator. RAW 264.7 cells were seeded into 96-well culture plates at  $2 \times 10^5$  cells/mL and stimulated with 1 μg/mL of LPS in the presence or absence of the

metabolites. Quercetin was used as positive control. After incubation at 37 °C for 24 h, Griess reagent I (50  $\mu$ L) of and Griess reagent II (50  $\mu$ L) were added to test the NO production. The absorbance was recorded at 550 nm against a calibration curve with sodium nitrite standards. Cell viability of the remaining cells was determined by the MTT method [9].



**Figure 1.** Compounds 1–8 from the marine fungus *Penicillium* sp. LPFH-Q3

### 3. Results and Discussion

#### 3.1. Structure Elucidation

HRESIMS analysis of compound **1** in positive mode revealed a protonated molecular ion at  $m/z$  377.1959  $[M + H]^+$ , consistent with the molecular formula  $C_{21}H_{28}O_6^+$  (calcd. 377.1959), requiring 8 double-bond equivalence. The  $^1H$  NMR spectrum showed the resonances for a methyl singlet ( $\delta_H$  1.29), two methyl doublets ( $\delta_H$  1.63, 1.02), five olefinic protons ( $\delta_H$  6.35, 6.26, 5.86, 5.50, 5.43) including two for a *cis* and two for a *trans* double bonds, three oxygenated protons ( $\delta_H$  5.38, 3.95 $\times$ 2), and several alkyl protons ( $\delta_H$  3.07, 2.11, 1.97, 1.84). The  $^{13}C$  NMR spectrum exhibited 21 carbon resonances. The carbons, aided by HSQC spectrum, were classified into six olefinic carbons ( $\delta_C$  162.0, 133.3, 130.8, 129.1, 126.6, 125.4) for three double bonds, three carbonyl carbons including an ester carbon ( $\delta_C$  172.9) and a carboxylic group ( $\delta_C$  177.2), three methyl carbons ( $\delta_C$  18.4, 18.0, 10.4), four methylene carbons ( $\delta_C$  61.5, 38.5, 34.6, 28.1) including an oxygenated, four  $sp^3$  methine carbons ( $\delta_C$  69.2, 40.7, 43.3, 47.5) containing an oxygenated, and one  $sp^3$  quaternary carbon ( $\delta_C$  36.5). Six of the eight degrees of unsaturation were occupied by three double bonds and three carbonyl carbons, the remaining two required that **1** was bicyclic. These spectroscopic data were similar to those of acrememophilane B, an eremophilane-type sesquiterpene isolated from an *Acremonium* sp. fungus [10].

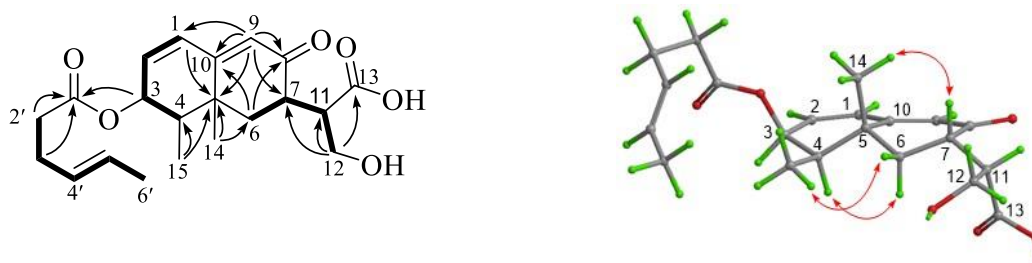
The  $^1H$  and  $^{13}C$  NMR data in combination with the 2D NMR data established the structure (Figure 2). The vicinally coupled protons at  $\delta_H$  6.35 (d,  $J = 9.7$  Hz) and 6.26 (dd,  $J = 5.2, 9.7$  Hz) located a *cis* double bond at C-1 and C-2, while the HMBCs between olefinic proton H-9 ( $\delta_H$  5.83) and C-1 ( $\delta_C$  130.8), C-5 ( $\delta_C$  36.5), C-7 ( $\delta_C$  43.3), and C-8 ( $\delta_C$  200.0) positioned an  $\alpha,\beta$ -unsaturated ketone moiety at C-8, C-9 ( $\delta_C$  125.4) and C-10 ( $\delta_C$  162.0). A 3-hydroxypropanoic acid unit was connected to C-7 ( $\delta_C$  43.3) by the COSY relationship between H-11 ( $\delta_H$  3.11) with H-7 ( $\delta_H$  3.07) and H-12 ( $\delta_H$  3.95)

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in combination with the HMBCs from H<sub>2</sub>-12 to C-7, C-11 ( $\delta_{\text{C}}$  47.5), C-13 ( $\delta_{\text{C}}$  177.2).

**Table 1.** <sup>1</sup>H (400 Hz) and <sup>13</sup>C NMR (100 Hz) Data of **1** ( $\delta$  in ppm) in CDCl<sub>3</sub>.

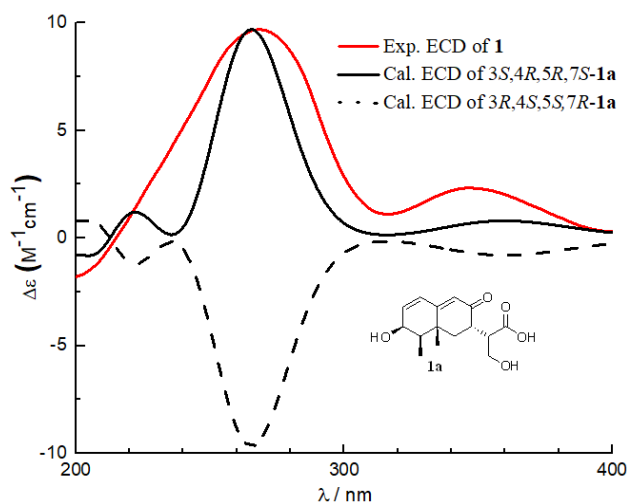
No.	$\delta_{\text{C}}$	$\delta_{\text{H}}$	No.	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	130.8, CH	6.35, d (9.7)	12	61.5, CH <sub>2</sub>	3.95, br s
2	133.3, CH	6.26, dd (9.7, 5.2)	13	177.2, C	
3	69.2, CH	5.38, dd (5.2, 4.6)	14	18.4, CH <sub>3</sub>	1.32, s
4	40.7, CH	1.97, m	15	10.4, CH <sub>3</sub>	1.05, d (7.0)
5	36.5, C		1'	172.9, C	
6	38.5, CH <sub>2</sub>	$\beta$ 2.11, dd (12.7, 4.0) $\alpha$ 1.84, dd (12.7, 12.7)	2'	34.6, CH <sub>2</sub>	2.43, m
7	43.3, CH	3.07, m	3'	28.1, CH <sub>2</sub>	2.35, m
8	200.0, C		4'	129.1, CH	5.43, m
9	125.4, C	5.86, s	5'	126.6, CH	5.50, m
10	162.0, C		6'	18.0, CH <sub>3</sub>	1.66, d (5.9)
11	47.5, CH	3.11, m			



**Figure 2.** Key <sup>1</sup>H-<sup>1</sup>H COSY (—), HMBC (---), and NOESY correlations (↔) of **1**

The COSY correlations from H<sub>2</sub>-2' ( $\delta_{\text{H}}$  2.43) to H<sub>3</sub>-6' ( $\delta_{\text{H}}$  1.66) via H<sub>2</sub>-3' ( $\delta_{\text{H}}$  2.35) and the olefinic protons H-4' ( $\delta_{\text{H}}$  5.43) and H-5' ( $\delta_{\text{H}}$  5.50), as well as the HMBCs from H<sub>2</sub>-2' and H<sub>2</sub>-3' to the ester carbonyl carbon C-1' ( $\delta_{\text{C}}$  172.9) assigned a 4'-hexenoic acid unit. Finally, the three-bond HMBC between H-3 to the ester carbonyl carbon permitted us to establish the attachment of 4'-hexenoic acid unit to the eremophilane nucleus at C-3 ( $\delta_{\text{C}}$  69.2).

A detailed comparison of the NOESY data and *J* values between compounds **1** and acemeremophilane **B** revealed that they shared the same relative configuration. The NOESY correlations from H<sub>3</sub>-14 ( $\delta_{\text{H}}$  1.32) to H<sub>3</sub>-15 ( $\delta_{\text{H}}$  1.05) and H-7 ( $\delta_{\text{H}}$  3.07) and between H-4 ( $\delta_{\text{H}}$  1.97) and H-6 $\alpha$  ( $\delta_{\text{H}}$  1.84) demonstrated the  $\alpha$ -orientation of H-4 and H-6 $\alpha$  and  $\beta$ -orientation of H<sub>3</sub>-14, H<sub>3</sub>-15, and H-7, and H-4 was in axial orientation. The *J*<sub>H-3/H-4</sub> value (4.6 Hz) reflected an equatorial-axial relationship of H-3 and H-4, revealing the same orientation of H-3 and H-4. Thus, the relative configuration of **1** was assigned to be 3*S*\*, 4*R*\*, 5*R*\*, and 7*S*\*.



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

The absolute configuration of compound **1** was determined by comparison of experimental of **1** and TDDFT-calculated ECD spectra for the model molecule of **1a** (Figure 3). The similarity of the theoretical spectrum of **3S,4R,5R,7S-1a** with the experimental spectrum of **1** indicated that compound **1** had a **3S,4R,5R,7S**-configuration. Compound **1** was given the trivial name penicisepene.

Additionally, the other compounds were identified to be guignarderemophilane E (**2**) [11], 6-desmethylmonacolin-J (**3**) [12], ( $\beta$ R, $\delta$ R,1S,2S,8S,8aR)-1,2,6,7,8,8a-hexahydro- $\beta,\delta,8$ -trihydroxy-2-methyl-1-naphthaleneheptanoic acid (**4**) [13], viridicatol (**5**) [14], viridicatin (**6**) [15],  $\beta$ -indoleacetic acid (**7**) [16], cyclo(L-pro-L-ile) (**8**) [17] based on comparison of the NMR data with those of the assigned structures published in literature. The NMR data of **3** and **4** in methanol- $d_4$  were reported for the first time.

**Table 2.**  $^1\text{H}$  (400 Hz) and  $^{13}\text{C}$  NMR (100 Hz) data of **3** and **4** ( $\delta$  in ppm) in  $\text{CD}_3\text{OD}$

No.	<b>3</b>		<b>4</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	37.8	1.81, m	37.8	1.81, m
2	32.2	2.38, m	32.2	2.39, m
3	133.4	5.71, m	133.7	5.71, dd (6.2, 3.6)
4	129.9	5.93, d (9.5)	129.8	5.92, d (9.8)
4a	135.0		135.0	
5	124.7	5.49, brs	124.5	5.48, s
6	21.6	2.38, m	21.6	2.07, m
		2.19, m		2.39, m
7	36.6	1.96, m	30.6	1.94, m
8	65.1	2.19, m	65.2	1.66, m
8a	39.8	4.22, m	40.0	4.22, m
9	34.0	1.48, m	25.0	2.18, m
		1.76, m		1.34, m
10	25.0	1.48, m	35.5	1.81, m
				1.55, m
11	78.4	4.71, m	71.3	3.8, m
12	30.6	1.96, m	44.8	1.41, m
		1.75, m		1.66, m
13	63.3	3.68, s	68.5	4.18, m
14	39.1	2.73, m	43.7	2.49 d (9.8)
		2.54, m		
15	173.8			
2-Me	14.3	0.89, d (7.0)	14.3	0.89 d (7.1)

$^1\text{H}$ -NMR (400 MHz),  $^{13}\text{C}$ -NMR (100 MHz),  $J$  in Hz,  $\delta$  in ppm

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## 3.2. Inhibitory Effects Toward NO production in LPS-activated RAW 264.7 Macrophages

First, the cell viability was evaluated by MTT method to evaluate the cytotoxicity of compounds on RAW 264.7 cells at 50  $\mu\text{M}$ . The results indicated that compounds **1–8** were non-toxic with more than 90% cell survival. Subsequently, all compounds were tested for the inhibitory effects on NO production at 25.0  $\mu\text{M}$ , the results showed that compounds **1, 3, 4** showed significant inhibition rate more than 70%, while other compounds exhibited inhibition rate below 30%, the  $\text{IC}_{50}$  values of **1, 3**, and **4** were determined (Table 3). The activities of compounds **1–4** revealed that the ester units in **1** and **3** were important for the activity, since the activity of **2** was negligible, and compound **3** was more active than its lactone-ring-opening derivative **4**.

**Table 3.** Inhibitory effects of the compounds **1–8** on NO production.

No.	Inhibition (%) <sup>a</sup>	$\text{IC}_{50}$ ( $\mu\text{M}$ )
<b>1</b>	86%	13.2
<b>2</b>	16%	nt. <sup>b</sup>
<b>3</b>	91%	10.6
<b>4</b>	74%	17.9
<b>5</b>	23%	nt. <sup>b</sup>
<b>6</b>	18%	nt. <sup>b</sup>
<b>7</b>	9%	nt. <sup>b</sup>
<b>8</b>	11%	nt. <sup>b</sup>
Quercetin		15.6

<sup>a</sup> at 30  $\mu\text{M}$ , <sup>b</sup> not tested

## Supporting Information

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

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