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A New Highly Oxygenated Isocoumarin from the Fungus *Penicillium* sp. Hzw-Fp1

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Abstract: *Penicillium* is a significant source of bioactive compounds, with the well-known antibiotics penicillin and griseofulvin derived from *P. chrysogenum* and *P. griseofulvum*, respectively. In our study, the fungus *Penicillium* sp. Hzw-Fp1 was isolated from shallow-sea sediments. The ethyl acetate extract of this strain exhibited moderate inhibitory effects against *Escherichia coli*. Subsequent isolation and purification of the extract led to the identification of five compounds (**1**–**5**), including a new highly oxygenated isocoumarin and two analogues (**2** and **3**), as well as two furan derivatives (**4** and **5**). The structure determination of compound **1** was conducted by extensive analysis of spectroscopic data, including ^{1}H and ^{13}C NMR, as well as 2D NMR techniques (HSQC, COSY, HMBC, and NOESY), in addition to HRESIMS. The known compounds **2**–**4** were identified as decarboxydihydrocitrinone (**2**), phenol A acid (**3**), 5-(hydroxymethyl)furfural (**4**), and 2-furoic acid (**5**) by comparing their ^{1}H and ^{13}C NMR data with those reported in the literature. Compound **1** exhibited a MIC value of 64 μg/mL toward *Escherichia coli.*

Keywords: *Penicillium* sp.; isocoumarin; fungus; isolation; structure elucidation.

1. Fungus Source

The strain Hzw-Fp1 was isolated from sea sediments collected along the Hangzhou Bay coastline. It was identified as *Penicillium* sp. through a comparison of the ITS region of its rDNA sequence with entries in the GenBank. The strain is identical to a known strain (MN521825.1) in the GenBank database (http://www.ncbi.nlm.nih.gov).

2. Previous Studies

The ocean presents an extreme ecological environment, where marine organisms have developed unique metabolic pathways to adapt to harsh conditions. This adaptation has resulted in the production of compounds with unprecedented structural frameworks and significant biological properties. Since over 70% of the Earth′s surface is covered by oceans, marine organisms provide a vast and diverse resource of structurally varied compounds. Marine fungi have been proven to be an important source of a majority of these compounds [1]. In recent years, there has been a notable

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increase in the search for bioactive molecules derived from marine fungi, particularly species of *Penicillium* [2, 3]. Studies have shown that the secondary metabolites of *Penicillium* strains are characterized by their structural diversity and unique bioactivities, offering a wealth of candidates for the development of pharmaceuticals. Recent chemistry research resulted in the identification of numerous compounds, including the 16-membered macrocyclic polyketides berkeleylactones [4], novel meroterpenoids meroantarctines [5], new tanzawaic acid derivatives [6, 7], interconvertible pyridone alkaloids [8], chromone derivatives [9], trienoic acid derivatives [10], indole alkaloid [11], and isocoumarin [12].

In our study, we isolated the strain *Penicillium* sp. from shallow-sea sediments, and its ethyl acetate (EtOAc) extract demonstrated significant inhibitory effects against *Escherichia coli*. Various chromatographic separations of the extract yielded five compounds, including a new highly oxygenated isocoumarin (**1**), two analogues (**2** and **3**), and two furan derivatives (**4** and **5**) (Figure 1). The isolation, structural determination, and bioactivity of these compounds are described herein.

3. Present Study

The fermentation process was conducted in 15 Fernbach flasks, each with a capacity of 500 mL. Each flask was added 80 g of rice and 90 mL of distilled water. The rice and water mixture were soaked for 6 hours prior to autoclaving in a steam sterilizer at 121 °C for 15 min. The strain was cultivated on potato dextrose agar (PDA) medium at room temperature for 4 days at 28°C in an incubator. After this initial growth period, a small piece of the medium containing the purified fungus was transferred under sterile conditions to the rice medium. Subsequently, the flasks were incubated at room temperature for 30 days. The culture medium was extracted with EtOAc for three times. The organic phase was concentrated under vacuum using a rotary evaporator to give a crude (1.1 g). The crude extract was subjected to a silica gel eluted with the solvent petroleum ether (PE)/acetone (30:1→2:1) to yield ten fractions (Fr.a–Fr.j). Fr.d was subjected to ODS C-18 eluted with MeOH/H_2O (30%→60%) to give **4** (2.1 mg) and **5** (1.6 mg). Fr.e was purified using Sephadex LH-20 eluted with MeOH to give **2** (6.7 mg). Fr. g was purified on HPLC using the eluent MeOH/H_2O (37:63) to afford **3** (t_R 11.3 min, 4.6 mg) and **1** (t_R 17.9 min, 3.2 mg).

*4β-Hydroxyembeurekol A (**1**):* Colorless oil; $[\alpha]^{25}_D$ +12 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 261 (3.47), 212 (4.11) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 279.0479 $[M + Na]^+$ (calcd. for $C_{11}H_{12}O_7Na^+$, 279.0475).

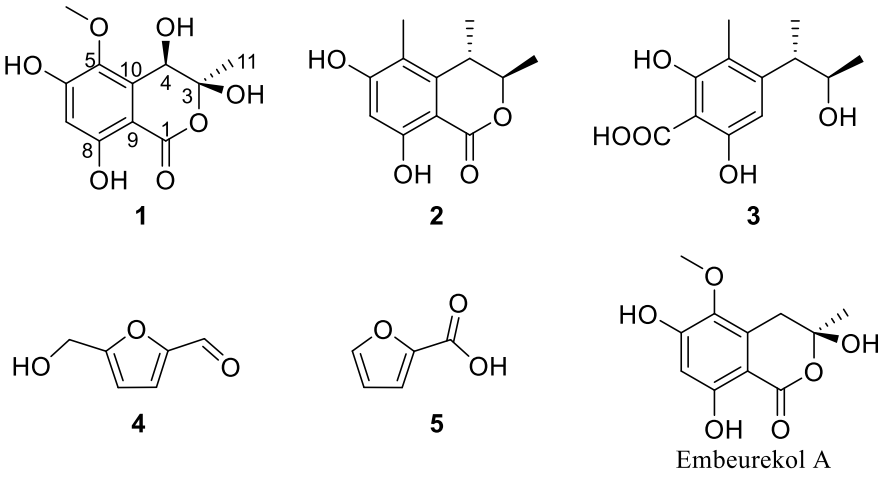

Figure 1. Chemical structures of secondary metabolites **1**–**5** from the fungus *Penicillium* sp. Hzw-Fp1

The molecular formula of compound **1** was determined to be $C_{11}H_{12}O_7$ based on the strong peak at *m/z* 279.0479 in the HRESIMS spectrum, which indicates six degrees of unsaturation. The ^{1}H NMR spectrum displayed a methyl singlet at δ_H 1.61, an oxygenated methine proton at δ_H 4.49 (1H, d, *J* = 6.1 Hz), a methoxy group at δ_H 3.71 (s), and four hydroxy protons (δ_H 5.86, 7.51, 10.69, and 11.0), as assigned with the assistance of the HSQC spectrum.

The ^{13}C NMR and HSQC spectra revealed a total of 11 carbon resonances, including six aromatic carbons (δ_C 99.0, 103.3, 134.5, 139.1, 158.5, 159.2) for a benzene ring, an ester carbonyl carbon (δ_C 169.1), an oxygenated methine carbon (δ_C 64.0), and a hemiketal or ketal carbon (δ_C 105.2). The presence of the benzene ring and the ester carbonyl carbon revealed five degrees of unsaturation, suggesting that there was an additional ring in the structure.

These data were very similar to those of embeurekol A [13], an isocoumarin isolated from the fungus *Embellisia eureka*. The only difference was due to the presence of an oxygenated methine (δ_H 4.49, δ_C 64) in **1** and the absence of the methylene CH_2 (δ_H 3.18, 3.10, δ_C 32.2) in embeurekol A.

Table 1. ^{1}H and ^{13}C NMR Data of **1** and Embeurekol A in DMSO-d_6 [a]

No.	**1**		**Embeurekol A**	
	δ_H	δ_C	δ_H	δ_C
1		169.1		168.2
2				
3		105.2		105.1
4	4.49, d (6.1)	64.0	3.18, d (16.9) 3.10, d (16.9)	32.2
5		139.1		137.8
6		158.5		158.3
7	6.37, s	103.3		101.6
8		159.2	6.29, s	159.1
9		99.0		98.5
10		134.5		130.9
11	1.61, s	24.3	1.62, s	22.3
3-OH	7.51, s			
4-OH	5.86, d (6.1)			
5-OCH_3	3.71, s	61.6	3.63, s	60.2
6-OH	10.69, s		10.7, s	
8-OH	11.0, s		10.9, s	

[a] ^{1}H NMR recorded at 400 MHz, ^{13}C NMR recorded at 100 MHz.

This suggested that compound **1** was the 4-hydroxylated derivative of embeurekol A, which was confirmed by the HMBC correlations (Figure 2) from the the methyl protons H_3-11 to C-4 (δ_C 64.0), from the O-bearing proton H-4 at δ_H 4.49 to C-9 (δ_C 99.0), C-10 (δ_C 134.5), along with the ^{1}H-^{1}H COSY correlation between H-4 and HO-4 (δ_H 5.86) (Figure 2). The structure of **1** was further secured by detailed analysis of the 2D NMR data (Figure 2).

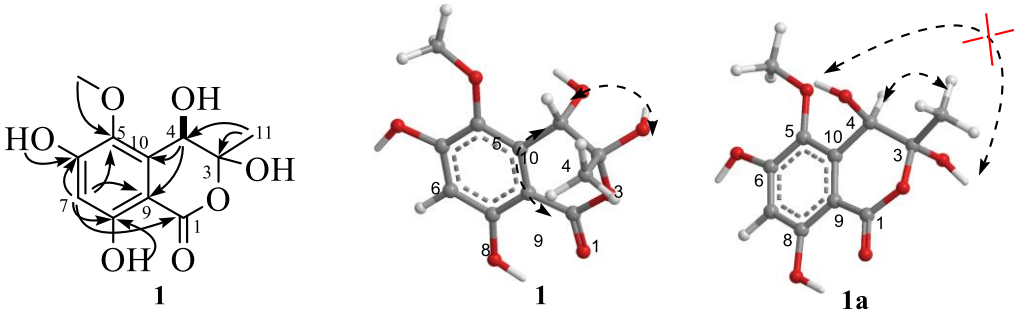

Figure 2. Key ^{1}H-^{1}H COSY (—) and HMBC correlations (⤳) of **1**, NOESY (⇢) correlations of **1** and its epimer **1a**

The relative configuration of **1** was determined by analyzing the NOESY data (Figure 2). Specifically, the NOESY correlations between H_3-11 (δ_H 1.61) and H-4 (δ_H 4.49), as well as between OH-3 (δ_H 7.51) and OH-4 (δ_H 5.86) revealed that the CH_3-11 and H-4 were in the same orientation, while the hydroxy groups were in the opposite direction. Besides, in the epimer **1a**, there will be no NOESY correlation between OH-3 and OH-4 due to the long distance between these protons (**1a** in Figure 2). Thus, the gross structure of **1** was determined as depicted.

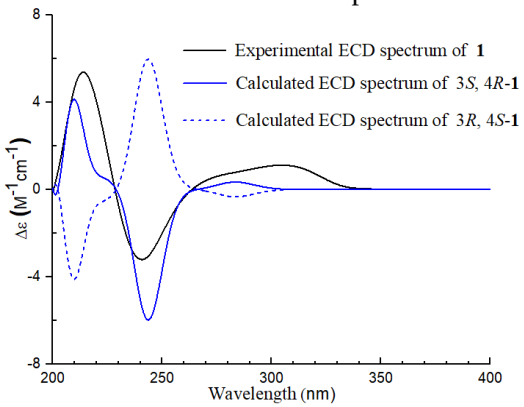

Figure 3. The experimental ECD spectrum of **1** and the calculated ECD spectrum of 3*S*, 4*R*-**1** and the enantiomer 3*R*, 4*S*-1

To determine the absolute configuration of the two chiral carbons C-3 and C-4, the theoretical ECD spectra of 3*S*,4*R*-**1** and 3*R*,4*S*-**1** were calculated. The calculated spectrum of 3*S*,4*R*-**1** exhibited a similar ECD curve to the experimental ECD curve of **1** (Figure 3), with obvious negative Cotton effect around 245 nm and a positive Cotton effect around 215 nm. Thus, the absolute configuration of C-3 and C-4 in **1** was assigned as *S* and *R*, respectively. Compound **1** was named 4β-hydroxyembeurekol A.

It is worth noting that the NMR data for compound **1** closely resemble those of the recently reported natural product ascoisocoumarin A (the C-3 epimer of **1**) [14]. The relative configuration of ascoisocoumarin A was determined by comparing the calculated ^{13}C NMR data of the two possible epimers with the experimental ^{13}C NMR data, with no regard to the NOE correlations between the two alcoholic hydroxy protons. Thus, the relative configuration may have been incorrectly assigned. Furthermore, the observed Cotton effects in the CD spectrum of ascoisocoumarin A were negligible, suggesting that ascoisocoumarin A was obtained as a racemic mixture. Since the calculated ECD spectrum for (3*S*, 4*R*)-**1** in our study was very similar to that for (3*R*, 4*R*)-ascoisocoumarin A [14], we can conclude that the chiral center at C-4 contributes significantly more to the strength of the Cotton effects than the chiral center at C-3.

Furthermore, the remaining four isolated metabolites were identified to be decarboxydihydrocitrinone (**2**) [15], phenol A acid (**3**) [16], 5-(hydroxymethyl)furfural (**4**) [17], and 2-furoic acid (**5**) [18] on the basis of the nearly identical NMR data compared to those bearing the same gross structures in the literature

All the five compounds were tested for their inhibitory effects against *Escherichia coli* ATCC 25922, only compound **1** exhibited an MIC value of 64 μg/mL, while other compounds were inactive at a concentration of 256 μg/mL**.**

Supporting Information

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

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