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Two New Compounds Isolated from A Seaweed-associated Fungus, *Aspergillus* sp. AF044

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[ABSTRACT] AIM: To study the chemical constituents of the seaweed fungus *Aspergillus* sp. AF044. **METHODS:** The fermentation extract was isolated and purified by column chromatography (MPLC, *Sephadex* LH-20, normal Si gel). **RESULTS:** Three compounds (**1** - **3**) were isolated and determined as 6-hydroxy-5-methoxy-3-methyl-3, 6-dihydro-2*H*-pyran-4-carboxylic acid (**1**), 8, 9-dihydroxy-8, 9-deoxyaspyrone (**2**) and penicillic acid (**3**) based on their spectral data (^{1}H, ^{13}C, HMQC, HMBC, ^{1}H-^{1}H COSY and NOE). **CONCLUSION: 1** and **2** are new compounds; Penicillic acid was isolated from many fungi.
[KEY WORDS] *Aspergillus* sp. AF044; Spectral data; Aspyrone

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1 Introduction

A growing number of marine fungi have been reported to produce novel and potentially useful bioactive secondary metabolites [1-2]. In recent years, numerous novel compounds discovered from marine fungi have been reported [1, 3].

As a part of ongoing effort to discover biologically active metabolites from the seaweed microorganism, *Asperigullus* sp. AF044 was selected from our screening program for further studies to examine the antimicrobial activity of its extract against *Candida albicans* ATCC 10231 and ATCC AS2.538. This paper describes the isolation and characterization of compounds **1**-**3** and their antibacterial effects against tested microbe.

2 Apparatus and Material

2.1 *Apparatus and material*

Column chromatography (CC) silica gel: Qingdao (200-300 mesh); Merck silica gel 60 Rp-18; Sephadex LH-20: Phar- macia products; TLC: Qingdao precoated plates, silica GF_{254} plates; NMR Spectra: Bruker DRX-600 spectrometer with TMS as internal standard; HRQTOF mass spectrometer; in *m*/z: Bruker BioTOF-Q.

2.2 *Isolation and fermentation of the strain.*

The strain AF044 was isolated from the seaweed collected from Jinjiang Dongshi Salt Field and was identified as *Aspergillus* sp. according to its morphological properties. The strain grows well on PDA media with aerial hyphae and yellow vegetative hyphae and produce lots of conidiospores on conidial head. The strain was inoculated on slope of PDA media in a test tube and cultivated for 5 d at 28 °C to afford seed cultures. Solid-state fermentation (1 L) was performed with PDA media for 15 d at 28 °C.

2.3 *Extraction and isolation*

The fungal strain AF044 was fermented and was extracted three times with equal volume of AcOEt/MeOH/AcOH 80∶15∶5 (*V*/*V*/*V*) at room temperature. The organic solutions were collected by filtration and the solvents removed under vacuum at 40 °C to yield crude extract (1.19 g).

The extract (1.19 g) was subjected to MPLC (80 g Rp-18 silica gel; H_2O, acetone/H_2O 30%, 50%, 70%, acetone, 1 L respectively) to afford nine fractions (Fr. 1 - 9). Fr. 1(166 mg) was subjected to Sephadex LH-20 (80 g; MeOH) to yield 4 fractions: Fr. 1A-1D. Fr. 1D (54.8 mg) was further purified by CC (*Sephadex LH-20,* 40 g, Acetone) to yield **1** (30 mg) and Fr. 1D2 (3.8 mg). Fr. 1D2 (3.8 mg) was further purified by CC (SiO_2; $CHCl_3$/MeOH 100∶1, 80∶1) to afford **2** (2 mg).

Fr. 3 (465 mg) was subjected to Sephadex LH-20 (80 g; MeOH) to yield 3 fractions: Fr. 3A-3C. The crystal (**3**, 30 mg)

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was appeared from Fr. 3A and Fr. 3B in acetone and water.

3 Results and Discussion

3.1 *Structure elucidation and identification*

Compound **1** was isolated as yellow crystal. Its molecular formula was determined as $C_8H_{12}O_5$ based on its HR-Q-TOF-ESI-MS ion peaks at *m/z* 211.062 4 $[M + Na]^+$ and NMR data.

The ^{13}C NMR (DEPT) spectrum of **1** showed 8 signals: two Me, one CH_2, two CH (one oxygenated), two CH, and three quaternary carbons including one carbonyl at δ 171.7 and a carbon-carbon double band at δ 178.6 and 106.6 (Table 1). The ^1H NMR spectrum showed a methyl doublet at δ 0.70 (d, J = 7.0 Hz), one oxymethyl at δ 3.92 (H-3a), one oxymethine protons at δ 5.08, and one oxymethylene at δ 3.79 and 4.04. The ^1H-^1H COSY spectrum of **1** revealed the connectivity of carbons at 5-6-7. The HMBC correlation between H-3a (δ 3.92) and C-3 (δ 178.6) suggested that the OMe group was at C(3). The downshift of C-3 (δ 178.6), C-4 (δ 89.4) and C-5 (δ 64.7) revealed the presence of three oxy carbons besides C-3a. Further analysis of HMBC data (Table 1) connected all carbons. Two unsaturation degrees attributed to carbonyl, double band and another unsaturation degrees assigned as a six-membered epoxy ring (including carbon 4-3-2-6-5). Therefore, the skeletal structure of **1** was elucidated.

Table 1 The NMR data of compound 1 (at 600 and 150 MHz in $CDCl_3$, δ, J in Hz)

No.	^1H	^{13}C	HMBC
1	/	171.7s	/
2	/	106.6s	/
3	/	178.6s	/
3a	3.92 (s, 3H)	59.8q	C-3
4	5.08 (s, 1H)	89.4d	C-2, C-3, C-1 (weak)
5	3.79 (dd, 4.3, 10.4, 1H) 4.04 (t, 10.4, 1H)	64.7t	C-2, C-6, C-7
6	2.44 (m, 1H)	37.5d	C-2, C-5, C-7
7	0.70 (d, 7.0, 3H)	11.3q	C-2, C-5, C-6

The relative configuration of **1** was determined by analysis of the NOE spectrum. The presences of NOE correlations between H-C(5) and H-C(6), H-C(5) and H-C(7), H-C(7) and H-C(6) indicated that H-C(5), H-C(6), H-C(7) were at the same side and in β-orientation, while H-C(4) and H-C(1a) were in α-orientation (Fig. 1).

Compound **2** was isolated as yellow oil. Its molecular formula was determined as $C_9H_{14}O_5$ based on its HR Q-TOF MS ion peaks at *m/z* 203.096 2 $[M + H]^+$ and 241.224 8 $[M + K]^+$ and NMR data. Detailed analyses of the ^1H and ^{13}C NMR spectra of **2**, including DEPT, COSY, HMQC, and HMBC experiments, revealed signals ascribable to an α,β-unsaturated δ-lactone, having 1, 2-dihydroxypropyl, hydroxyl, and methyl substituents (Table 1). The ^1H-^1H COSY spectrum of **2** in $CDCl_3$ revealed the connectivity of carbons at 4-5-6-7 and 8-9-10. The analysis of HMBC data for **2** (Table 2) connected all carbons assigning the skeletal structure as shown in Fig 1. Those spectroscopic features revealed that compound **2** had the general structural features of aspyrone [4]. Again, NMR assignments were similar to aspyrone[4] and 9-chloro-8-hydroxy-8, 9-deoxyaspyrone[5] except for the chemical shift of C-8 and C-9. Thus, the structure of **2** was determined as 8, 9-dihydroxy-8, 9-deoxyaspyrone.

The relative stereochemistry of **2** was assigned by NOE and by an analysis of vicinal proton-proton coupling constants. The NOE correlation between H-5 and H_3-7 suggested that H-5 and H-6 were *trans* oriented, which was further supported by comparing the coupling constant between H-5 and H-6 in **2** ($J_{H5\text{-}H6}$ = 8.8 Hz) with values reported for the *trans* stereoisomers of aspyrone ($J_{H5\text{-}H6}$ = 8.5 Hz), and 9-chloro-8-hydroxy-8, 9-deoxyaspyrone ($J_{H5\text{-}H6}$ = 9.0 Hz)[5] and the *cis* stereoisomer of (+)-goniotriol ($J_{H5\text{-}H6}$ = 3.2 Hz)[6]; the NOE correlations between H-C(5) and H-C(8), and H-C(8) and H-C(9) suggested that H-C(8) and H-C(9) were *cis*- oriented.

Compound **3** was elucidated by comparison of the spectral data (^1H and ^{13}C NMR) with those reported in the literature[7] to be penicillic acid.

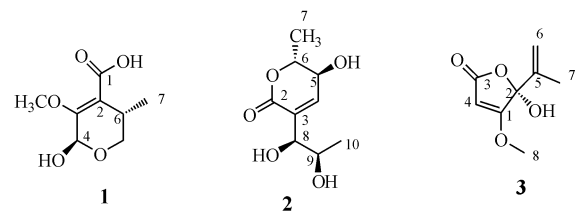

Fig. 1 Structures of compounds 1-3

Table 2 The NMR data of compound 2 (at 600 and 150 MHz in $CDCl_3$, δ, J in Hz)

No.	^1H	^{13}C	HMBC	^1H-^1H COSY
2	/	163.8s	/	/
3	/	130.7s	/	/
4	6.89 (s)	144.7d	C-2, C-8	H-5
5	4.30 (d, 8.8)	68.0d	C-3,C-6	H-4, H-6
6	4.37 (m)	78.8d	C-2, C-4	H-5, H-7
7	1.41 (d, 6.4)	17.9q	C-6, C-5	H-6
8	4.52 (d, 4.2)	73.1d	C-2, C-3, C-4, C-9	H-9
9	4.12 (q, 6.2)	69.1d	C-3	H-8, H-10
10	1.16 (d, 6.2)	17.8q	C-8, C-9	H-9

4 Discussion

From 1 L fermentation extract, two new (**1** - **2**) and one known (**3**) compounds were isolated. Aspyrone was isolated as a nematicidal compound from *Aspergillus melleus* [4], and chlorine containing aspyrone-derivatives isolated from *As-*

pergillus ostianus showed antibacterial activity against *Ruegeria atlantica* and *E. coli* [5]. Penicillic acid also showed activity against *R. atlantica*. In our study, three compounds showed no inhibitory activity against *Candida albicans* ATCC 10231 and AS 2.538 at concentration 50 μg per disc.

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海藻真菌菌株 *Aspergillus* sp. AF044 中的两个新天然产物

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【摘　要】 **目的：**研究海藻真菌 *Aspergillus* sp. AF044 的化学成分。**方法：**通过色谱层析柱对提取物进行分离纯化, 并通过波谱解析(一维、二维的核磁共振谱和质谱)确定化合物的结构。**结果：**分离纯化得到 3 个化合物, 分别鉴定为 6-hydroxy-5-methoxy-3-methyl-3, 6-dihydro-2*H*-pyran-4-carboxylic acid (**1**), 8, 9-dihydroxy-8, 9-deoxyaspyrone(**2**) and penicillic acid(**3**)。**结论：**化合物 **1** 和 **2** 是首次发现的新化合物。化合物 **3** 在多种真菌菌株中分离得到。

【关键词】 *Aspergillus* sp. AF044; 光谱数据; Aspyrone

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