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A New Sesquiterpenoid Derivative from the Coastal Saline Soil Fungus *Aspergillus fumigatus*

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Abstract: A new sesquiterpenoid derivative, named aspergiketone (**1**), along with seven known compounds (**2**-**8**) were isolated from the coastal saline soil fungus *Aspergillus fumigatus*. Their structures were elucidated by spectroscopic analysis, and by comparison of experimental and reported data. The absolute configuration of compound 1 was defined by X-ray diffraction analysis. Compound **1** was cytotoxic towards HL-60 and A549 cell lines with IC_{50} values of 12.4 and 22.1 μM, respectively.

Keywords: secondary metabolites; *Aspergillus fumigatus*; cytotoxic activity; coastal saline soil.

1. Introduction

Aspergillus fumigatus is widely distributed in various environments, such as mouldy corn, polluted oil or food, marine organisms, etc [1]. *A. fumigatus* is a fungus well known to produce chemically and biologically diverse metabolites. Over the last few decades, at least 226 potentially bioactive secondary metabolites have been reported, which can be divided into 24 biosynthetic families including the fumigatins, fumigaclavines, fumiquinazolines, trypacidins, monomethylsulochrins, fumagillins, gliotoxins, pseurotins, chloroanthraquinones, fumitremorgins, verruculogens, helvolic acids, and pyripyropenes, etc [2-4]. These structure diversed secondary metabolites exhibited a wide range of biological activities, such as antitumor and antibacterial. In addition, they played an important role in diseases caused by *A. fumigatus* [5-6]. We focused on the secondary metabolites of *A. fumigatus* isolated from coastal saline soil collected in Wudi, China in August 2011. Preliminary studies showed that the culture extract of the strain had strong cytotoxic activity against brine shrimp larvae with an IC_{50} value of 5.8 μg/mL. Through repeated silica gel column chromatography, Sephadex LH-20 column chromatography, and semi-preparative HPLC, eight compounds (Figure **1**) were identified: aspergiketone (**1**), fumiquinazoline C (**2**) [7],

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fumitremorgin C [8], cyclotryprostatins A (**4**) [9], fumiquinazoline B (**5**) [10], demethoxyfumitremorgin C (**6**) [11-12], tryprostatin A (**7**) [13], and pseurotin A (**8**) [13-14]. The cytotoxic activities against HL-60 and A549 cell lines of compound **1** were evaluated by the MTT method.

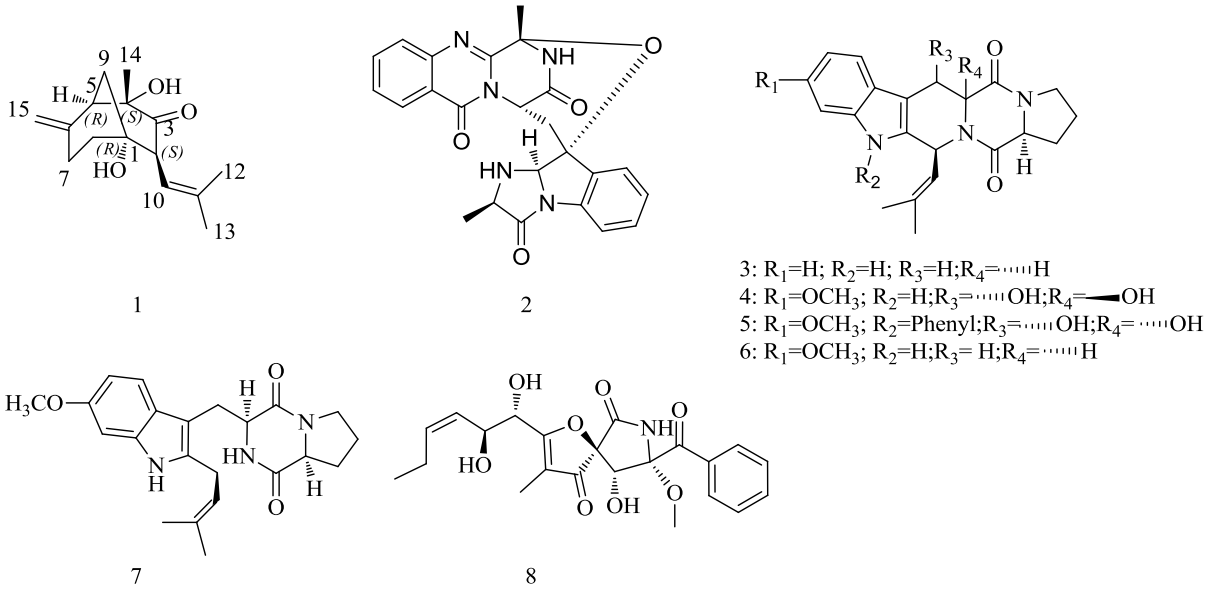

Figure 1. Structures of compounds **1**-**8**

2. Materials and Methods

2.1.General

UV spectra were recorded on a TU-1091 spectrophotometer. IR spectra were taken on a Nicolet 6700 spectrophotometer using Attenuated Total Reflection (ATR) method. NMR spectra were recorded on a Bruker AV-400 spectrometer using TMS as internal standard. HR-ESI-MS data were recorded on a Q-TOF Ultima GLOBAL GAA076 LC mass spectrometer. Single crystal diffraction spectra were obtained on a Bruker SMART CCD 1000 X-ray diffractometer. Semipreparative HPLC was performed on a SHIMADZU LC-6AD Liquid Chromatograph with SPD-20A Detector, using an ODS column [HyperClone 5 μm ODS (C_{18}) 120A, 250 × 10 mm, Phenomenex, 4 mL·min^{-1}]. All cell lines were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China).

2.2. Microorganism Material

The strain of *A. fumigatus* was isolated from marine mud collected from coastal saline soil in Wudi, Shandong province, China in August 2011 and the working strain was identified on the basis of sequence analysis of the ITS region of the 16S rDNA. A voucher specimen was deposited in our laboratory with the number LDS20110826AF.

2.3. Fermentation and Isolation

Spores were directly inoculated into 500-mL Erlenmeyer flasks containing 180 mL fermentation media (glucose 20 g, maltose 10 g, mannitol 10 g, yeast extract 3 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, dissolved in 1 L sea water). The cultures were incubated at 28 ℃ on a rotary shaker at 165 rpm for 7 days. The whole broth (20 L) was filtered through cheesecloth to separate the broth supernatant and mycelia. The former was extracted with ethyl acetate, while the latter was extracted with CH_3OH. The CH_3OH extract was evaporated under reduced pressure to afford an aqueous solution, and then

extracted with ethyl acetate. The two ethyl acetate extracts were combined and concentrated under reduced pressure to give a crude extract (22 g).

The crude extract was subjected to silica gel CC, eluted with $CHCl_3$-CH_3OH (100∶1→0∶1) to give 12 fractions (*Fr1-Fr12*). Compound **1** was obtained from *Fr5* as colorless block crystals (9.0 mg). Compound **8** (40 mg) was obtained as a white precipitate from *Fr6*. *Fr6* was further separated on Sephadex LH-20 column chromatography with CH_3OH, and compound **2** (14 mg) was obtained as white crystals. *Fr7* was separated on a silica gel column using $CHCl_3$-CH_3OH (50:1, v/v) as mobile phase into four subfractions (*Fr7.1- Fr7.4*), and compounds **3** (10 mg) and **4** (11 mg) was obtained from *Fr7.2* and *Fr7.3* as white precipitates, respectively. *Fr8* was separated by silica gel CC over silica gel using $CHCl_3$-CH_3OH (50:1, v/v) as mobile phase, to afford compounds **5** (12 mg) and **7** (9 mg). *Fr9* was separated on a Sephadex LH-20 column eluted with methanol, to give six subfractions (*Fr9.1-Fr9.6*). *Fr9.3* was purified by RP-HPLC using CH_3OH-H_2O (45:55), to afford compound **6** (8 mg, retention time was 14 min).

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as colorless block crystals (m.p. 87.5 ℃) in methanol. The molecular formula of **1** was determined to be $C_{15}H_{22}O_3$ with five indices of hydrogen deficiency by HR-ESI-MS (*m*/*z* 249.1497 $[M - H]^-$, cald. 249.1496) and NMR data (Table 1). The IR spectra showed absorptions for hydroxyls (3433 and 3321 cm^{-1}), carbonyl (1699 cm^{-1}), and olefinic (1651 cm^{-1}) functionalities. The ^1H NMR data (Table 1) displayed the presence of three methyl proton signals [δ 1.03 (3H, *s*), 1.54 (3H, *s*), 1.75 (3H, *s*)], four methylenes [δ 2.06 (1H, *dd*, *J* = 15.4, 5.4 Hz), 1.60 (1H, *dd*, *J* = 15.4, 5.4 Hz); 1.84 (1H, *m*), 1.23 (1H, *m*); 2.67 (1H, *d*, *J* = 12.0 Hz), 1.51 (1H, *m*); 4.69 (1H, *s*, *brs*), 4.74 (1H, *s*, *brs*)], three methines [δ 3.75 (1H, *d*, *J* = 9.4 Hz), 2.56(1H, *brs*), 5.18 (1H, *d*, *J* = 9.4 Hz)] and two hydroxyl protons. The analysis ^{13}C NMR spectra showed 15 signals corresponding to one carbonyl carbon (δ 210.0), four olefinic carbons (δ 146.5, 134.9, 118.3 and 111.2), two oxygenated tertiary carbon atoms (δ 73.6 and 73.3), two methines (δ 55.5 and 52.4), three methylenes (δ 39.3, 35.5), and 28.8), three methyls (δ 26.1, 21.7, and 18.5). One carbonyl group and two pairs of double bonds accounted for three indices of hydrogen deficiency, and the remaining two indices indicated the presence of two of ring systems in compound **1**. Further interpretation of the HMBC data showed correlations from H-C (12) to C (10), C (11) and C (13), H-C (13) to C (10), C (11) and C (12), indicative of the presence of an isobutenyl unit (fragment A, Figure **2**) in compound **1**. Similarly, the HMBC correlations from H-OH (1) to C (1), C (2), C (8) and C (9), H-C (2) to C (1), C (3), C (8) and C (9), H-C (14) to C (3), C (4) and C (5), H-OH (4) to C (3), C (4), C (5), C (6) and C (14), H-C (5) to C (3), C (4), C (5) and C (9), H-C (9) to C (1), C (2), C (3), and C (8), H-C (8) to C (1), C (2) and C (9) established the bicyclic moiety B (Figure **2**). Fragment C (Figure **2**) was also deduced via the HMBC correlations from H-C (15) to C (5), C (6) and C (7), H-C (7) to C (15). The HMBC correlations from H-C (2) to C (10) and C (11) suggested that the isobutenyl was located at C (2). Also, the HMBC correlations from H-C (5) to C (6), H-C (9) to C (6) established the C (6)-C (5) linkage. According to the indices of hydrogen deficiency, there should be another ring indicative of the C (7)-C (8) linkage. Therefore, the 2D structure of **1** was tentatively assigned as shown in Figure **1**.

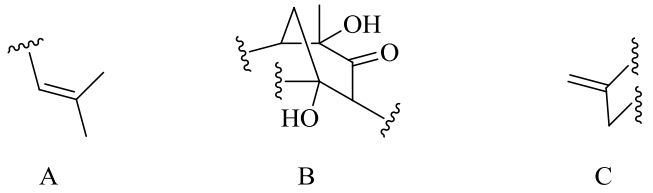

Figure 2. Fragments A-C

However, compound **1** contained four stereogenic carbon centers: C (1), C (2), C (4), and C (5). Two of these centers lacked protons, which severely hindered the NMR-based absolute configurational assignments. These problems were solved by single crystal diffraction X-ray analysis using CuKα radiation, and the resulting ORTEP drawing is presented in Figure **3**. Based on the crystallographic analysis, the absolute configuration of compound **1** was defined as 1*R*, 2*S*, 4*S*, 5*R*. Thus, compound **1** was given the trivial name, aspergiketone. Crystallographic data of **1** have been deposited in the Cambridge Crystallographic Data Center (no. CCDC 0001000242420). These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. This is the first report of a sesquiterpenoid derivative from *A. fumigatus*.

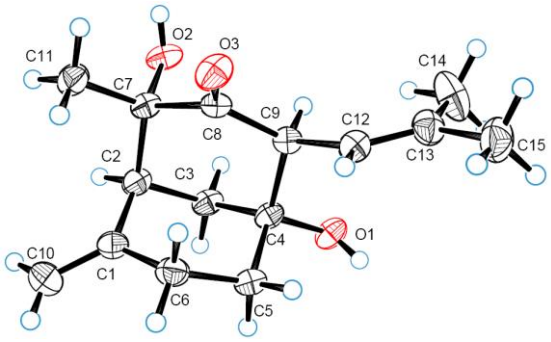

Figure 3. ORTEP drawing of the crystal structure of compound **1**

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of Compound **1** (in DMSO-d_6, in *ppm*, *J* in Hz) and Diagnostic HMBC cross peaks.

Position	δ_H	δ_C	HMBC
1		73.6	
2	3.75 (1H, *d*, *J* = 9.4)	55.5	C-1, C-3, C-8, C-9, C-10, C-11
3		210	
4		73.3	
5	2.56（1H, *brs*)	52.4	C-3, C-4, C-6, C-9
6		146.5	
7	2.06 (1H, *dd*, *J* = 15.4, 5.4) 1.60 (1H, *dd*, *J* = 15.4, 5.4)	28.8	C-15
8	1.84 (1H, *m*) 1.23 (1H, *m*)	35.5	C-1, C-2, C-9
9	2.67 (1H, *d*, *J* = 12.0) 1.51 (1H, *m*)	39.3	C-1, C-2, C-5, C-6, C-8
10	5.18 (1H, *d*, *J* = 9.4)	118.3	
11		134.9	
12	1.54 (3H, *s*)	18.5	C-1, C-3, C-10, C-11, C-13
13	1.75 (3H, *s*)	26.1	C-1, C-3, C-10, C-11, C-12
14	1.03 (3H, *s*)	21.7	C-3, C-4, C-5
15	4.74 (1H, *s*, *brs*) 4.69 (1H, *s*, *brs*)	111.3	C-5, C-6, C-7
1-OH	4.46 (1H, *s*)		C-1, C-2, C-8, C-9
4-OH	5.58 (1H, *s*)		C-3, C-4, C-5, C-6, C-14

*Crystal data and structure refinement for compound **1***: The molecular formula of compound **1** was $C_{15}H_{22}O_3$, fw = 255.33, orthorhombic space group $P2_12_12_1$, unit cell dimensions a = 6.72260(10) Å, b = 11.26120(10) Å, c = 19.1305(2) Å, V = 1448.27(3) Å^3, $\alpha = \beta = \gamma = 90°$, Z = 4, ρ_{calc} =1.148 mg/mm^3, crystal size was 0.36 × 0.32 × 0.26 mm, θ range for data collection was 4.56 to 69.58°, T =

150(2), μ = 0.628 mm^{-1}, F(000) = 544, Goodness-of-fit on F^2 = 1.050. The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques (SHELXS-97). The final cycle of full-matrix least-square refinement was based on 20173 unique reflections and 168 variable parameters and converged with unweighted and weighted agreement factors of R1 = 0.0265, and wR2 = 0.0677 [I>2σ (I)].

3.2. Cytotoxicity activity

Compound **1** was subjected to cytotoxic evaluation against HL-60 and A-549 cell lines employing the MTT method [15]. Cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 ℃.

Compound **1** exhibited a cytotoxicity against HL-60 and A-549 cells with IC_{50} values of 12.4 and 22.1 μM, respectively.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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