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Polyketides and Alkaloids from the Deep-Sea-Derived Fungus *Aspergillus fumigatus CBC18132*

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Abstract: The fungal strain *Aspergillus fumigatus* CBC18132, isolated from deep-sea sediment, was investigated for secondary metabolites. Fermentation on rice medium followed by chromatographic separation led to the isolation of three polyketides (**1**–**3**) and ten alkaloids (**4**–**13**). The structures were determined by analyses of spectroscopic data (^{1}H and ^{13}C NMR, and MS data). The absolute configuration of the anthraquinone-derivative trypacidin (**1**) was resolved for the first time by a combination of ECD and specific rotation calculation. The probable biogenetic relationships of compounds **1**–**3** were described. All isolated compounds were inactive toward the α-glucosidase at the initial concentration of 4 mM.

Keywords: *Aspergillus fumigatus*; trypacidin; absolute configuration.

1. Plant Source

The fungal strain CBC18132 was isolated from the sediments that were collected from the Western Pacific (DY48I-BC1813) at a depth of -5270.29 m. The strain was identified as *Aspergillus fumigatus* by comparing the morphological characteristics and analysis of the ITS region of the rDNA sequence with those of standard record (LC388872.1). The ITS sequence has been deposited in GenBank (http://www.ncbi.nlm.nih.gov) with the accession number ON026087. The strain CBC18132 was deposited at the Marine Culture Collection of China (MCCC 3A01558).

2. Previous Studies

The fungus *A. fumigatus*, widely distributes in natural environment, has been proved to be a prolific species of producing secondary metabolites with conspicuous bioactivity or complex structures,

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among which the polyketides and alkaloids occupy a special place. In recent years, marine-derived *A. fumigatus* was frequently obtained and studied, leading to the identification of alkaloids (e.g. fumiquinazolines, secofumitremorgins, cephalimysins) [1–4], polyketides [5–7], terpenoids (e.g. the tetracyclic triterpenes helvolic acid derivatives) [8, 9], some exhibited pronounced biological activities, such as the polyketide penicillixanthone A, which exhibited remarkable anti-HIV-1 activity with IC_{50} values less than 0.5 μM [5].

In our study of secondary metabolites of deep-sea-derived fungi [10–13], the ^{1}H NMR spectrum of the EtOAc extract of the strain *A. fumigatus* displayed NMR signals that suggested the presence of polyketides and alkaloids. Extensive chromatographic separation of the fermentation resulted in the identification of thirteen known compounds, including three polyketides (**1**–**3**) and ten alkaloids (**4**–**13**). Herein, the isolation and structural identification of the metabolites were described, particularly, the absolute configuration of the anthraquinone-derivative trypacidin (**1**) was resolved for the first time in the current study.

3. Present Study

The solid-state fermentation was carried out in 55 erlenmeyer flasks (500 mL) with 80 g of rice and artificial sea-water (100 mL), and the contents were autoclaved at 15 psi for 30 min. Each flask was inoculated with 3.0 mL of the spore inoculum and incubated at room temperature for 35 days. The fermented materials were extracted with EtOAc (3 × 2000 mL) and gave an EtOAc extract (6.4 g), which was subjected to MCI column chromatography eluted with MeOH/H_2O (20:80 to 100:0) to afford four fractions (Fr.I–IV). Fra. IV was chromatographed over ODS silica gel CC (MeOH/H_2O = 20:80 to 100:0) to give ten fractions (Fr.A–Fr.J). Fr.I was purified on HPLC (a YMC-pack ODS-A column was used for semipreparative HPLC separation, 3 mL/min) using 50% acetonitrile/water as eluent to give **3** (t_R = 32.5 min, 4.7 mg) and **13** (t_R = 37.5 min, 8.7 mg). Fr.G was applied to the ODS silica gel CC (MeOH/H_2O = 20:80 to 100:0) to give five fractions (Fr.G1–Fr.G5). Fr.G5 was purified on HPLC using MeCN/H_2O (46:54) as a mobile phase to obtain **6** (t_R = 48.0 min, 14.1 mg) and **11** (t_R = 25.5 min, 6.8 mg). Fr.G4 was separated by HPLC (MeCN/H_2O = 47:53) to yield **2** (t_R = 47.0 min, 4.5 mg). Fr.B was purified on HPLC using MeCN/H_2O (24:76) as a mobile phase to obtain Fr.B1–Fr.B7 and **4** (t_R = 53.1 min, 26.1 mg). Fr.B1 was purified on HPLC (MeOH/H_2O = 43:57) as a mobile phase to afford **12** (t_R = 23.0 min, 3.9 mg). Fr.B4 was purified on HPLC using MeOH/H_2O (40:60) as a mobile phase to obtain **5** (t_R = 34.0 min, 5.4 mg). Compound **1** (15.6 mg) was precipitated from Fr.F. The rest of Fr.F was purified on HPLC (MeCN/H_2O = 35:65) as a mobile phase to obtain **7** (t_R = 65.0 min, 63.6 mg), **8** (t_R = 46.0min, 3.3mg), and **9** (t_R = 57.0 min, 6.5 mg). Fr.E was purified on HPLC (MeCN/H_2O = 42:58) to obtain six fractions (Fr.E1–Fr.E6), compound **10** (2.7 mg) was precipitated from Fr.E5.

*Trypacidin A (**1**)*: Colorless oil, $[\alpha]^{25}_D$ −176.5; ECD (*c*, 1.5 × 10^{-4} M) λ_{max} ($\Delta\varepsilon$) 218 (+27.33), 259 (−12.93), 283 (+9.87), 301 (−9.17) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z*: 345.0953 $[M + H]^+$ (calcd. for $C_{18}H_{17}O_7^+$, 345.0969).The NMR and MS data of compounds **2**–**13** were listed in the supporting information.

Compound **1** was isolated as a colorless oil, its molecular formula was established to be $C_{18}H_{16}O_7$ by HRESIMS at *m/z* 345.0953 $[M + H]^+$ (calcd.. 345.0969; mean error: −4.64 ppm). The ^{1}H NMR spectrum provided the resonances for three methoxys [δ_H 3.94 (3H, s), 3.68 (3H, s), 3.65 (3H, s)] including an aromatic one [3.94 (3H, s)], four aromatic or olefinic protons [δ_H 7.10 (1H, d, *J* = 1.6 Hz), 6.54 (1H, s, H-7), 6.37 (1H, s, H-5), 5.76 (1H, d, *J* = 1.6 Hz)], and an aromatic methyl [δ_H 2.43 (3H, s)]. The ^{13}C NMR spectrum resolved 18 carbon resonances that were attributable to three carbonyl carbons (δ_C 190.6, 185.8, 163.6), four sp^2 methine carbons (δ_C 105.6, 105.5, 104.0, 137.2), six sp^2 non-protonated carbons, four methyl carbons including three methoxy carbons, and one sp^3 non-protonated carbon (δ_C 84.2) with the aid of HSQC spectrum. The above-mentioned structural features were quite similar to those of the known compound trypacidin, which was first isolated from the same species in 1963. Detailed analyses of the 2D NMR data (Figure 2) and comparisons of the spectroscopic data with those of trypacidin led to the identification of **1** to be trypacidin. [14, 15]

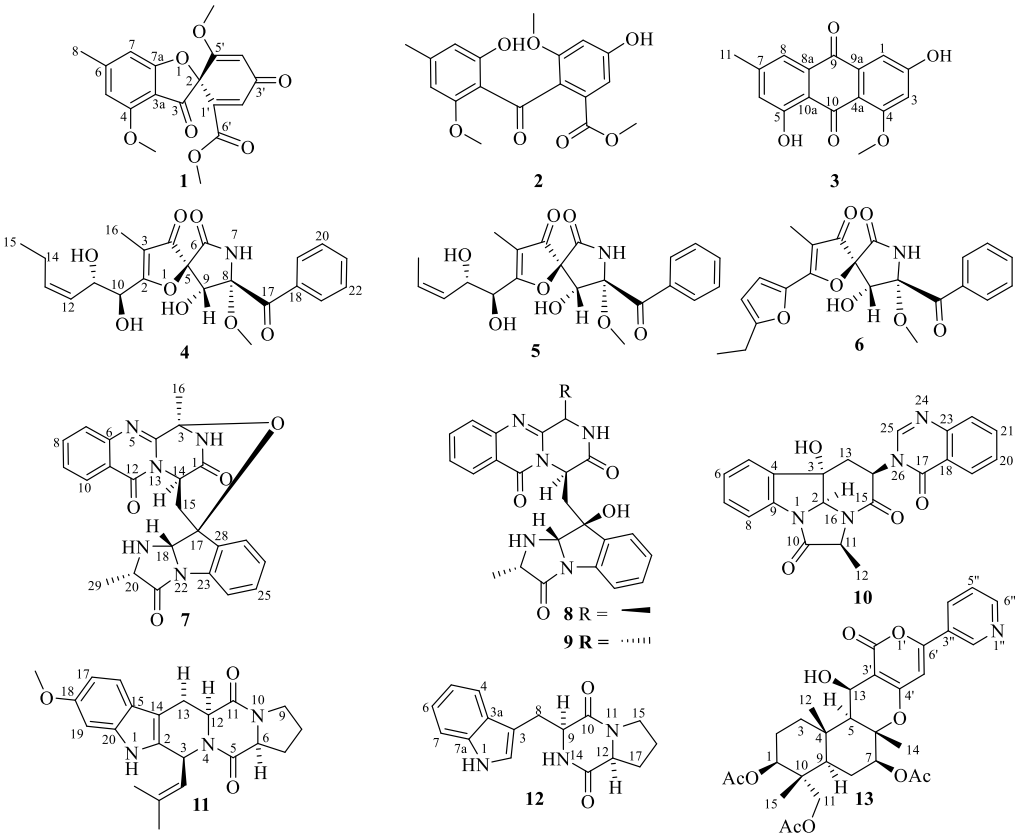

Figure 1. Structures of compounds **1**–**13** from *A. fumigatus* CBC18132

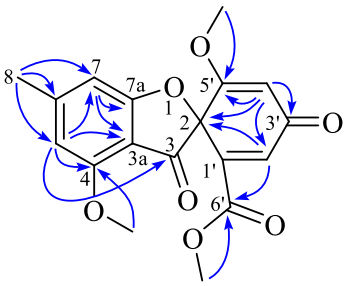

Figure 2. Key HMBC (⟶) correlations of **1**

The gross structure of trypacidin was reported for dozens of times up to now, while the absolute configuration of the only chiral center of C-2 was still left unresolved. In order to determine the absolute configuration of **1**, the experimental and calculated ECD data were compared (Figure 3). On basis of the TDDFT-ECD method, the ECD data of the model compound (*R*-1) were calculated at the b3lyp/6-31+g(d,p) level with the solvation model density (SMD) in methanol using the b3lyp/6-31+g(d,p) optimized two conformers (C1 and C2) after conformational searches via the MMFF94S force field. Theoretical ECD spectrum of the corresponding enantiomer (*S*-**1**) was obtained by directly inverse of the calculated ECD spectrum of *R*-1. Comparison of the experimental ECD data of **1** with the calculated spectra indicated **1** to be in agreement with the 2*S* configuration.

Table 1. ^{1}H (400 Hz) and ^{13}C NMR (100 Hz) Data of **1**–**3** (δ in ppm)

No.	**1**[a]		**2**[b]		No.	**3**[c]	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C
1					1	7.20, d (2.2)	107.00
2		84.0		141.9	2		164.52
3		190.4		201.1	3	6.84, d (2.2)	105.00
3a		108.3		129.9	4		163.46
4		158.3		162.6	4a		112.64
5	6.37, s	105.5	6.21, s	104.7	5		
6		152.1		149.6	6	7.12, d (1.2)	124.18
7	6.54, s	105.5	6.39, s	108.7	7		146.63
7a		174.3		165.3	8	7.43, d (1.7)	119.12
8	2.43, s	23.1	2.29, s	22.4	8a		132.05
1'		138.2		132.6	9		182.33
2'	7.09, d (1.6)	137.0	6.68, d (2.2)	111.4	9a		136.82
3'		185.6		158.5	10		186.33
4'	5.76, d (1.6)	103.9	6.69, d (2.2)	104.1	10a		114.39
5'		169.4		159.7	11	2.39, s	21.37
6′		163.4		168.0	4-OCH_3	3.90, s	56.34
4-OCH_3	3.94, s	56.0	3.38, s	56.3			
5′-CH_3	3.65, s	56.7	3.69, s	56.5			
6′-OCH_3	3.68, s	52.7	3.66, s	52.5			

[a] in $CDCl_3$, [b] in methanol-d_4,[c] in DMSO-d_6

In addition, the comparable experiment specific rotation ($[\alpha]^{25}_D$ −176.5) of **1** with the calculated data for the two possible enantiomers ($[\alpha]^{25}_D$ +217.9 for *R*-1 and $[\alpha]^{25}_D$ −217.9 for *S*-1) at the b3lyp/6-31+g(d,p) level with the SMD in MeOH provided additional evidence to support the configurational assignment (Table 2). Thus, the absolute configuration of C-2 in **1** was determined to be *S*.

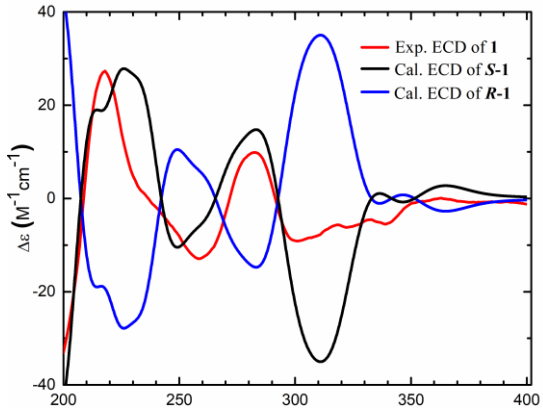

Figure 3. Experimental and calculated ECD spectra of **1** in MeOH

Compounds **1**–**3** are biogenetically related compounds (Figure 4), according to the literature [16, 17], the keto group at C-10 in questin (**3**) was reduced to a hydroxyl group by the reductase GedF with the aid of NADPH. The C-9−C-8a bond of questin hydroquinone (**3a**) is cleaved by the atypical cofactor-free dioxygenase GedK to yield desmethylsulochrin (**3b**), which was subsequently methylated by two methyltransferases (TpcM and TpcH) to afford monomethylsulochrin (**2**). An internal nucleophilic substitution in **2** catalyzed by the enzyme TpcJ gives trypacidin.

Besides, the other known compounds were established as monomethylsulochrin (**2**) [18], questin (**3**) [19], pseurotines A1 (**4**) [20], 14-norpseurotin A (**5**) [21], spirofuran A (**6**) [22], fumiquinazolines C (**7**) [2],(−)-fumiquinazoline B (**8**) [2], (−)-fumiquinazoline A (**9**) [2], chaetominine(**10**) [2], fumitremorgin C (**11**) [23], brevianamide F (**12**) [23], and pyrenampine A (**13**) [24] by comparing their ^{1}H and ^{13}C NMR data with reported data in the literature.

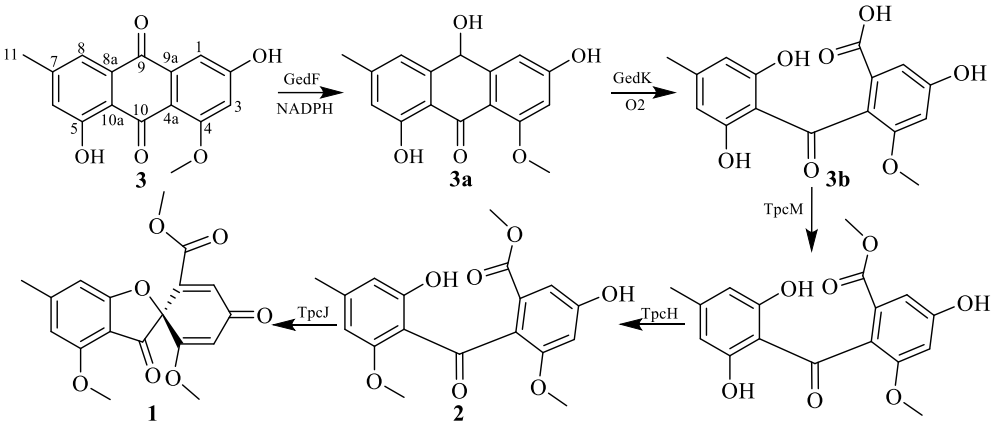

Figure 4. Biogenetic relationships of compounds **1**–**3**

All compounds were evaluated for their inhibitions toward the α-glucosidase at the initial concentration of 4 mM [25], while the inhibition rates of compounds **1**–**13** were all below 30%.

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Supporting Information

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