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NOTE

Indole alkaloids from marine-derived fungus *Aspergillus sydowii* SCSIO 00305

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Marine microorganisms have proved to be an important source of pharmacologically active metabolites, and a growing number of marine-derived fungi have been reported to produce metabolites with unique structures and interesting biological activities.[1,2] The genus *Aspergillus* (Moniliaceae), with over 180 species, has attracted considerable attention as a rich source of alkaloids, terpenoids, xanthones, polyketides and etc, some of which showed antifungal, antibacterial, anti-HIV and cytotoxic activities.[3–5] In order to obtain new bioactive metabolites from marine fungi, we investigated on the marine fungal strain *Aspergillus sydowii* SCSIO 00305 isolated from a healthy tissue of *Verrucella umbraculum*. Bioassay-guided fractionation led to the isolation of a new indole diketopiperazine alkaloid, cyclotryprostatin E (**1**), together with nine known ones, [4-(2-methoxyphenyl)-1-piperazinyl][(1-methyl-1H-indol-3-yl)]-methanone (**2**), cyclotryprostatin B (**3**),[6] fumiquinazoline D (**4**),[7] fumitremorgin B (**5**),[8] fumiquinazoline C (**6**),[7] fumiquinazoline B (**7**),[7] fumiquinazoline A (**8**),[7] fumiquinazoline F (**9**),[7] fumiquinazoline G (**10**)[7] from a culture broth of the strain. The structures of compounds (**1**) and (**2**) were characterized by spectroscopic data interpretation. Compound (**2**) was a synthetical compound, however, no reference for it. The NMR data and biology source of (**2**) were reported for the first time. We present herein the fermentation, isolation, structure elucidation and cytotoxicity of compounds (**1**) and (**2**).

Compound (**1**) was obtained as pale yellow powder with the molecular formula $C_{23}H_{29}N_3O_6$ deduced from NMR spectra and positive HRESIMS (found 466.1953 $[M+Na]^+$, calculated 466.1954). The UV bands (221, 293 nm) and IR absorptions at 3400, 3312, 1664 and 1653 cm^{-1} indicated the presence of conjugated system, hydroxyl and carbonyl groups. The ^1H NMR spectrum of (**1**) showed two methyl groups (δ_H 1.34 and 1.47), two methoxyl groups (δ_H 3.41 and 3.83), and three aromatic protons [δ_H 7.46 (d, J=9.0 Hz, H-16), 6.75 (dd, J=2.5, 9.0 Hz, H-17), 6.95 (d, J=2.5 Hz, H-19)]. The ^{13}C and DEPT NMR spectra showed signals for 23 carbons, including two methyls (δ_C 29.1, 31.5), four methylene groups (δ_C 22.7, 30.9, 46.4 and 50.8), one oxygenated methine carbon (δ_C 77.6), two oxygenated quaternary carbons (δ_C 71.2 and 87.3), eight olefinic carbons, and two amide carbonyl groups (δ_C 167.8 and 169.0). These NMR data of (**1**) showed similarity to those of (**3**),[3] which suggested that (**1**) was an indole diketopiperazine alkaloid. A spin coupling system of H_2-7/H_2-8/H_2-9 in the ^1H-^1H COSY spectrum (Figure 1), combined with HMBC correlations from H-16 to C-14/C-18/C-20, from H-19 to C-15/C-17/C-18/C-20, from H-3/H_2-7 to C-5, and from H-6 to C-11 (Figure 1), further confirmed the suggestion. Comparison of ^1H- and ^{13}C NMR data of (**1**) with those of cyclotryprostatin B (**3**) revealed that a tri-substituted double bond (δ_C 123.5 and 137.9) in (**3**) was replaced by an oxygenated quaternary carbon (δ_C 71.2) and a methylene group (δ_C 50.8) in (**1**). In the HMBC spectrum (Figure 1), correlations from H_3-23/H_3-24/H-3 to C-21/C-22 suggested the assignment of C-21 (δ_C 50.8) and C-22 (δ_C 71.2). The relative stereochemistry of (**1**) was determined by the NOESY spectrum, the magnitude of ^1H-^1H COSY coupling constants, and comparison of the ^{13}C NMR data of (**1**) with those of (**3**). The observed NOE correlations between H-3 and H_3-24/H_2_21, between H_3-24 and H_2-21, and between H-6 and H-7α/H-8α, together with nearly identical carbon chemical shift of C-12 (δ_C 87.3 in (**1**) and 84.7 in (**3**)) and C-13 (δ_C 77.6 in (**1**) and 76.8 in (**3**)), indicated that (**1**) had the same relative configuration as **3**.

Compound (**2**) was isolated as pale yellow crystals with the molecular formula $C_{21}H_{23}N_3O_2$ deduced from HRESIMS (*m/z* at 350.1865 $[M+H]^+$, calc. 350.1869). The ^1H-, ^{13}C-, and DEPT NMR spectra displayed 18 carbon signals, including one conjugated carbonyl group (δ_C 168.9), nine methines, one methoxyl (δ_H 3.84, δ_C 56.0), one methyl (δ_H 3.84, δ_C 33.3), one methylene (δ_C 52.4), and five quaternary carbons. Three spin coupling systems (H-5/H-6/H-7/H-8, H-20/H-21/H-22/H-23, and H-13(17)/H-14(18)) deduced from ^1H-^1H COSY spectrum, together with HMBC correlations from H-5

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to C-3/C-7/C-9, from H-2 to C-3/C-4/C-9, and from H-8 to C-4/C-6 suggested the existence of one indolyl group, one piperazinyl group, and one 1,2-disubstituted phenyl group. One methyl group and one methoxyl group were deduced to be located at N-1 and C-19, respectively, from the observation of HMBC correlations from δ_H 3.84 (6H, s) to C-2/C-9/C-19. This information coupled with the key HMBC correlations from H-2, H-13 and H-17 to C-11 and from H-14 and H-16 to C-18 enabled us to establish the structure of (**2**) as shown in Figure 2. We proposal that chorismate acid may be a biogenetic precursor for (**2**).

Compounds (**1**) and (**2**) were screened for their cytotoxicity against A549 (lung cancer cell line), A375 (human melanoma cell line) and Hela (Human cervical carcinoma cell) cell lines, using the MTT method with *cis*-platin as positive control. Compound (**2**) showed significant cytotoxicity against A375 cell lines with IC_{50} (half maximal inhibitory concentration) value of 5.7 μM. Compound (**1**) had no obvious cytotoxicity towards the above mentioned three cell lines.

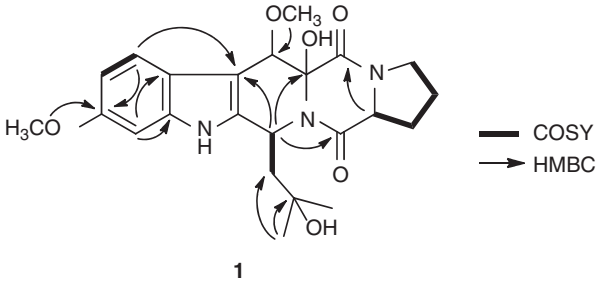
EXPERIMENTAL PROCEDURE

Taxonomy

The fungus *A. sydowii* SCSIO 00305 was isolated from a healthy tissue of *V. umbraculum* collected from Sanya, Hainan Province, China, and was identified by Dr Xiaoyong Zhang, and a voucher specimen (*A. sydowii* SCSIO 00305) has been deposited in the *RNAM Center for Marine Microbiology*, South China Sea Institute of Oceanology, Chinese Academy of Sciences.

Fermentation, isolation and identification of compounds

The fungus strain *A. sydowii* SCSIO 00305 was cultivated in 500 ml Erlenmeyer flasks containing 100 ml of the production medium composed of glucose 1%, maltose 2%, mannitol 2%, yeast extract 0.3%, monosodium glutamate 1%, $MgSO_4{\cdot}7H_2O$ 0.03%, KH_2PO_4 0.05% and sea water 5l (pH 7.2 before sterilization), and cultured without shaking at 28 °C for 20 days. The EtOAc extract of mycelia (800 mg) was chromatographed on RP-C 18 column using gradient elution from 5% MeOH/H_2O to100% MeOH, to give four fractions. Fraction A (MeOH/H_2O, 35% v/v elution, 100 mg) was further purified by semi-preparative reversed-phase HPLC (MeOH/H_2O 45 % v/v, 3 ml/min, detector 230 nm) to yield (**5**) (t_R=23.1 min), (**6**) (t_R=28.0 min), (**8**) (t_R=32.5 min). Fraction B (MeOH/H_2O, 55% v/v elution, 120 mg) was further purified by semi-preparative reversed-phase HPLC (MeOH/H_2O 50% v/v, 3 ml/min, detector 230 nm) to yield (**1**) (t_R=16.6 min), (**4**) (t_R=18.9 min), (**9**) (t_R=22.1 min) and (**10**) (t_R=23.8 min). Fraction C (MeOH/H_2O, 75% v/v elution, 250 mg) was further purified by reversed-phase HPLC (60%CH_3CN/H_2O, 3 ml/min, detector 254 nm) to yield (**2**) (t_R=12.8 min), (**3**) (t_R=15.2 min) and (**7**) (t_R=20.0 min).



1

Figure 1 Key correlations of COSY (bold line) and HMBC (arrow) of **1**.

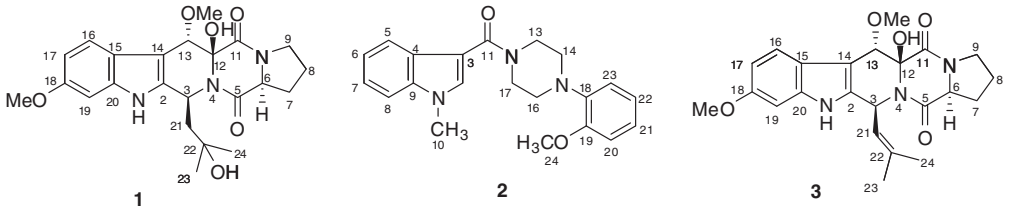
Cyclotryprostatin E (**1**): pale yellow powder; $[\alpha]_D^{25}$ +28.35 (*c* 0.23, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 209 (2.91), 251 (2.03); ^1H and ^{13}C NMR data see Table 1; IR (KBr) ν_{max} 3298, 2980, 2914, 1665, 1450, 1418, 1249, 1158, 1110, 1028 cm^{-1}; ESIMS *m/z* 466 $[M+Na]^+$; HRESIMS *m/z* 466.1953 $[M+Na]^+$, calculated for $C_{23}H_{29}N_3O_6Na$ *m/z* 466.1954).

[4-(2-methoxyphenyl)-1-piperazinyl](1-methyl-1H-indol-3-yl)-methanone (**2**): pale yellow powder; $[\alpha]_D^{25}$ +33.33 (*c* 0.06, CH_3OH); UV (CH_3OH) λ_{max}

Table 1 ^1H- and ^{13}C-NMR Data of 1 and 2[a]

	1		*2*	
No.	δ_H	δ_C	δ_H	δ_C
2	—	136.2	7.64 (s)	133.2
3	6.04 (dd, 6.0, 6.0)	49.0	—	110.1
4	—	—	—	127.7
5	—	167.8	7.73 (d, 8.0)	121.4
6	4.31 (dd, 6.0, 11.5)	60.7	7.22 (dd, 8.0, 8.0)	122.1
7α	2.46 (m)	30.9	7.29 (dd, 8.0, 8.0)	123.7
7β	1.90 (m)			
8α	2.11 (m)	22.7	7.47 (d, 8.0)	111.2
8β	2.04 (m)			
9α	2.22 (d, 6.2)	46.4	—	138.2
9β	2.18 (d, 6.2)			
10	—	—	3.84 (s)[b]	33.3
11	—	169.0	—	168.9
12	—	87.3	—	—
13	4.81 (s)	77.6	3.93[b]	/[c]
14	—	105.1	3.08[b]	52.4[b]
15	—	138.2	—	—
16	7.46 (d, 9.0)	119.3	3.08[b]	52.4[b]
17	6.75 (dd, 2.5, 9.0)	110.7	3.93[b]	/[c]
18	—	157.7	—	142.1
19	6.95 (d, 2.5)	96.1	—	154.0
20	—	123.7	6.98 (d, 8.0)	112.9
21	3.61 (m)	50.8	7.04 (dd, 8.0, 8.0)	125.0
	3.78 (m)			
22	—	71.2	6.92 (dd, 8.0, 8.0)	122.3
23	1.34 (s)	31.5	6.99 (d, 8.0)	119.8
24	1.47 (s)	29.1	—	—
CH_3O-13	3.41 (s)	57.3	—	—
CH_3O-18	3.83 (s)	56.1	—	—
CH_3O-19	—		3.84 (s)[b]	56.0

Abbreviations: d, doublet; dd, doublet of doubles; m, multiplet; s, singlet.
[a]At 500 and 125 MHz, respectively; δ in p.p.m., *J* in Hz, in CD_3OD.
[b]Overlapped signals.
[c]Not observed.



1 **2** **3**

Figure 2 Structures of **1**–**3**.

(log ε) 286 (3.84); ^{1}H and ^{13}C NMR data see Table 1; IR (KBr) ν_{max} 3428, 2932, 2831, 1612, 1534, 1500, 1471, 1434, 1238, 1154, 1139, 747 cm^{-1}; ESIMS *m/z* 466 $[M+Na]^+$; HRESIMS *m/z* 350.1871 $[M+H]^+$, calculated for $C_{21}H_{23}N_3O_2$ *m/z* 350.1868).

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