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New Quinazolinone Alkaloids within Rare Amino Acid Residue from Coral-Associated Fungus, *Aspergillus versicolor* LCJ-5-4

Yibin Zhuang, Xiancun Teng, Yi Wang, Peipei Liu, Guoqiang Li, and Weiming Zhu*

Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, Peoples' Republic of China

weimingzhu@ouc.edu.cn

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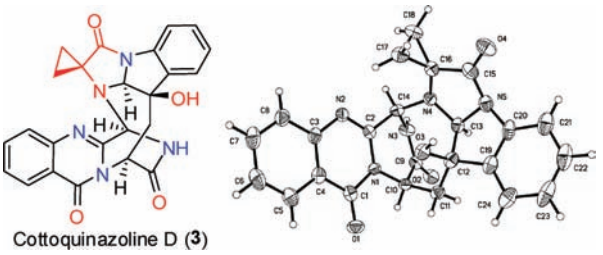
ABSTRACT



Cottoquinazoline D (3), a new alkaloid with a 1-aminocyclopropane-1-carboxylic acid residue rarely discovered in nature, was isolated and identified together with two new quinazolinone alkaloids, cottoquinazolines B (1) and C (2), from coral-associated fungus *Aspergillus versicolor* LCJ-5-4. Their structures including absolute configurations were elucidated based on spectroscopic methods, X-ray single crystal diffraction analysis, and chemical methods. A possible biogenetic pathway for them was proposed.

Coral is one of the three main sources of marine natural products,[1] from which various kinds of compounds with diverse bioactivities have been identified. For example, pseudopterosin A from *Pseudopterogorgia elisabethae* is now in phase II trials as a wound healing agent.[2] However, research on secondary metabolites of coral-associated microorganisms that were regarded as the true producers of their host-derived compounds have been rarely reported.[3] During our ongoing pursuit for structurally novel and bioactive natural products from microorganisms isolated from unusual or specialized ecological niches, we investigated the metabolites of 168 coral-associated fungal strains by integrated chemical and bioactive screening. Among these strains, a fungal strain LCJ-5-4 authenticated as *Aspergillus versicolor* was isolated from the soft coral *Cladiella* sp. collected from the South China Sea. About 150 secondary metabolites of *A. versicolor* were characterized, mainly including xanthones,[4] anthraquinones,[5] lactones,[6] chromones,[7] polyketides,[8] and tryptophane-derived alkaloids.[9,10] These compounds exhibited antimicrobial activity[8,11] and cytotoxicity.[9] The EtOAc extract of the fermentation broth of *A. versicolor* LCJ-5-4 showed

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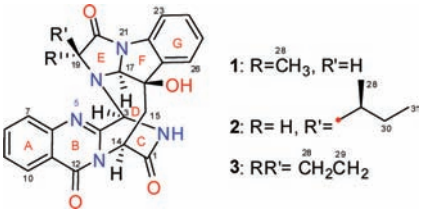
Table 1. ^{1}H and ^{13}C NMR Data for Cottoquinazolines B−D (**1**−**3**) (600, 150 MHz, DMSO-d_6, TMS, δ ppm)

	1		**2**		**3**	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	169.9, qC		169.8, qC		170.5, qC	
2		9.46, d (4.4)		9.46, d (5.0)		9.45, d (4.9)
3	64.4, CH	5.49, d (5.5)	63.9, CH	5.41, d (5.0)	63.0, CH	5.11, d (4.9)
4	149.4, qC		149.2, qC		150.3, qC	
6	147.2, qC		147.2, qC		147.4, qC	
7	127.4, CH	7.69, d (7.7)	127.3, CH	7.64, d (7.8)	127.4, CH	7.66, d (8.1)
8	134.7, CH	7.83, dt (7.7, 1.5)	134.7, CH	7.82, dt (7.8, 1.3)	134.8, CH	7.84, dt (7.7, 1.6)
9	127.0, CH	7.55, dt (7.7, 1.0)	126.9, CH	7.55, dt (7.8, 0.9)	127.1, CH	7.55, dt (7.7, 1.1)
10	126.4, CH	8.16, dd (7.7,1.5)	126.4, CH	8.16, dd (7.7, 1.3)	126.4, CH	8.16, dd (7.7, 1.6)
11	120.3, qC		120.3, qC		120.2, qC	
12	160.1, qC		160.1, qC		160.0, qC	
14	53.3, CH	5.37, d (6.5)	53.2, CH	5.38, d (6.8)	53.8, CH	5.34, d (6.6)
15	33.5, CH_2	3.11, dd (14.3, 6.6); 2.32, d (14.3)	33.6, CH_2	3.10, dd (15.1, 7.5); 2.31, d (14.6)	34.6, CH_2	3.13, dd (15.3, 6.6); 2.36, d (14.8)
16	78.6, qC		79.1, qC		75.8, qC	
16-OH		5.30, s		5.23, d (1.4)		5.40, s
17	82.1, CH	5.09, s	82.8, CH	5.07, t (1.4)	79.2, qC	5.16, s
19	59.2, CH	4.13, m	65.1, CH	4.19, s	50.4, qC	
20	174.1, qC		174.0, qC		167.1, qC	
22	139.0, qC		139.2, qC		138.9, qC	
23	117.5, CH	7.30, d (8.3)	118.3, CH	7.29, dd (8.2)	114.6, CH	7.32, d (7.8)
24	129.5, CH	7.31, dt (7.7, 1.1)	129.3, CH	7.30, dt (7.8, 1.4)	129.8, CH	7.33, t (7.7)
25	125.7, CH	7.16, dt (7.7, 1.5)	125.8, CH	7.16, dt (7.3, 1.3)	124.9, CH	7.11, dt (7.7, 2.2)
26	123.7, CH	7.29, d (7.8)	123.4, CH	7.26, d (8.2)	124.6, CH	7.36, d (7.5)
27	138.7, qC		139.0, qC		136.7, qC	
28	15.8, CH_3	1.49, d (6.6)	15.1, CH_3	1.07, d (6.8)	9.9, CH_2	1.54, m; 0.96, m
29			34.2, CH	2.11, m	7.4, CH_2	1.35, m; 1.15, m
30			25.0, CH_2	1.67, m; 1.56, m		
31			12.2, CH_3	1.04, t (7.3)		

significant cytotoxicity against P388 cells and different secondary metabolites from those reported by HPLC analysis with diode array detection. Chemical study resulted in the isolation and identification of three new tryptophane-derived quinazolinone alkaloids, named cottoquinazolines B−D (**1**−**3**). Compound **3** contained a 1-aminocyclopropane-1-carboxylic acid residue which has been rarely discovered in nature.



A. versicolor LCJ-5-4 was grown under static conditions at 20 °C for 30 days and then harvested by extraction with EtOAc. The extract (140 g) was separated by repeated silica gel vacuum liquid chromatography and HPLC purification to yield compounds **1** (5 mg), **2** (5 mg), and **3** (10 mg) (Supporting Information).[12]

Compound **1** was obtained as colorless prisms (MeOH), mp 273 °C (dec.), $[\alpha]_D^{25}$ +83 (*c* 0.5, MeOH). Its molecular formula was determined as $C_{23}H_{19}N_5O_4$ according to the HRESIMS at *m*/*z* 430.1526 $[M + H]^+$ (calcd for $C_{23}H_{20}N_5O_4$, 430.1515), requiring 17 degrees of unsaturation. The IR absorption bands at 3443, 3219, 1692, and 1670 cm^{-1} suggested the presence of hydroxy and amidocarbonyl groups. ^{1}H and ^{13}C NMR spectral analysis (Table 1) and literature retrieval revealed that **1** was the congener of cottoquinazoline A whose absolute configuration has not been assigned.[9] The main differences occurred in rings C−G, indicating a stereoisomer of cottoquinazoline A that was confirmed by 2D NMR (Figure 1). The relative configuration of **1** was assigned on the basis of NOE difference experiments. When H-3 and HO-16 were irradiated, the signals of H-17/28 and H-19 were enhanced by 8.7%, 5.3%, and 4.0%, respectively, indicating H-3/H-17/28 was *cis*-configuration and CH_3-28/HO-16 was *trans*-configuration that was opposite to cottoquinazoline A[9] (Figure 1). The X-ray diffraction experiment of **1** confirmed this

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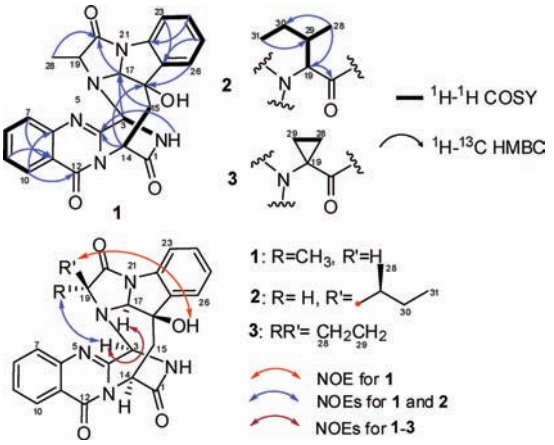

Figure 1. Key 2D NMR correlations and NOE effects of compounds **1**−**3**.

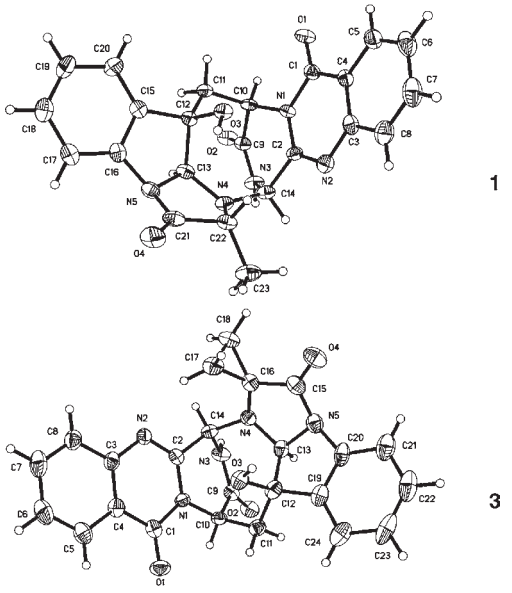

Figure 2. X-ray crystal structures of compounds **1** and **3**.

deduction and indicated a *cis*-configuration between H-3 and H-14 (Figure 2). Acidic hydrolysis of **1** afforded D-Ala that was identified by amino acid analysis with authentic samples (coinjection) on a chiral Crownpak CR(+) HPLC column (Figure S16).[13,14] Therefore, the absolute configuration of **1** was unambiguously assigned as (3*S*,14*R*,16*S*,17*S*,19*R*).

Compound **2**, was obtained as colorless prisms (MeOH), mp 263 °C (dec.), with a molecular formula of $C_{26}H_{25}N_5O_4$ from HRESIMS at *m*/*z* 472.1984 $[M + H]^+$ (calcd for $C_{26}H_{26}N_5O_4$, 472.1985), $[\alpha]_D^{25}$ +31 (*c* 1.0, MeOH). Except

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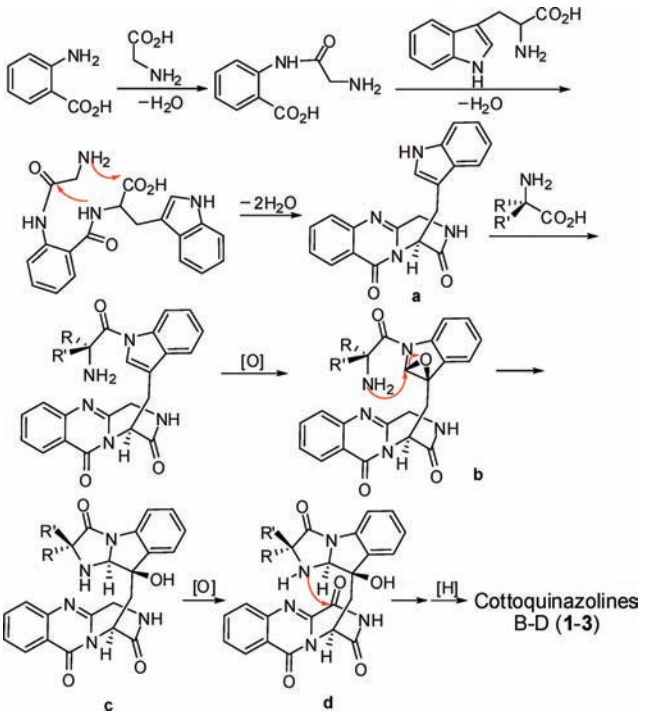
Scheme 1. Plausible Biogenetic Pathway of Quinazolinone Alkaloids



for an additional CH_3CH_2CH moiety, the NMR data (Table 1) were almost identical to those of **1**, indicating that **2** was the congener of **1** in which the alanine was replaced by the isoleucine. This inference was confirmed by ^{1}H−^{1}H and ^{1}H−^{13}C correlation spectroscopy experiments (Figure 1) and amino acid analysis. Acidic hydrolysis of **2** afforded L-Ile that was identified by the same method as that for **1** (Figure S17). The absolute configuration of **2** was thus determined to be (3*S*,14*R*,16*S*,17*S*,19*S*,29*S*) (Figure 1).

Compound **3** was obtained as colorless prisms (MeOH), mp 270 °C (dec.), with a molecular formula of $C_{24}H_{19}N_5O_4$ according to HRESIMS at *m*/*z* 442.1501 $[M + H]^+$ (calcd for $C_{24}H_{20}N_5O_4$, 442.1515), $[\alpha]_D^{25}$ +78 (*c* 0.2, MeOH). The similarity of NMR (Table 1) between **3** and **1** indicated **3** also as the congener of **1**. Comparison of NMR data revealed that two characteristic methylenes of cyclopropane ($\delta_{H/C}$ 1.54 and 0.96/9.9 and 1.35 and 1.15/7.4) and quaternary carbon (δ_C 50.4) in **3** replaced the methyl at $\delta_{H/C}$ 1.49/15.8 and methine at $\delta_{H/C}$ 4.13/59.2 in **1**, respectively. In addition, C-16, C-17, and C-20 were shifted upfield by 2.8, 2.9, and 7.0 ppm, respectively. These data supported that D-Ala residue in **1** was replaced by 1-aminocyclopropane-1-carboxylic acid residue that was confirmed by amino acid analysis. When hydrolyzed, **3** produced 1-aminocyclopropane-1-carboxylic acid that was identified on a chiral Crownpak CR(+) HPLC column by coinjection with an authentic sample (Figure S18).[13,14] An NOE difference experiment and X-ray diffraction revealed the same relative configuration

compared to those of **1** and **2**. Additionally, the specific rotation of **3** was of the same sign as those of **1** and **2**, suggesting that **3** also has the same (3*S*,14*R*,16*S*,17*S*)-configuration (Figure 1).

The new quinazolinone alkaloids **1**−**3** are probably biosynthesized *via* an amino acid pathway (Scheme 1). Intermolecular condensation among anthranilic acid, glycine, and tryptophan produced intermediate **a** that further condensed with the fourth amino acid, and the obtained product was oxidized to form the corresponding epoxidation product **b**. Intramolecular nucleophilic cyclization of **b** produced **c** that was oxidized to form **d**. The oxidation product **d** further underwent an intramolecular reductive amination to form compounds **1**−**3** (Scheme 1). Compounds **1**−**3** were evaluated in vitro for cytotoxicity against Hela and P388 cells using an MTT assay with 5-FU as a positive control[15] and for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Bacillus subtilis*, and *Candida albicans* by an agar dilution method.[16]

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Compound **3** exhibited moderate antifungal activity against *Candida albicans* with an MIC value of 22.6 μM, while other compounds did not show antimicrobial activity at a concentration of 100 μg/mL. Compounds **1**−**3** showed no cytotoxicity against the tested Hela and P388 cell lines ($IC_{50} > 50\ \mu M$).

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Supporting Information Available. The NMR spectra and UV and IR data of **1**−**3**, the X-ray data for **1** and **3**, HPLC profiles of acidic hydrolysates of **1**−**3**, 18S rRNA sequence of *A. versicolor* LCJ-5-4, isolation, purification, and bioassay protocols used. This material is available free of charge via the Internet at http://pubs.acs.org.