A New Polyketide from Marine-Derived 
*Paraconiothyrium* sp

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Abstract

A new polyketide, paraconthone A (I) was obtained from the fermentation culture of *Paraconiothyrium* sp., together with two known compounds (2-3). Their structures were elucidated by spectroscopic analyses, including 1D- and 2D-NMR experiments, HR-ESI-MS, ECD calculation and by comparison with reported data. Compounds 1 to 3 were evaluated for their cytotoxic, anti-inflammation and antimicrobial activities in vitro, and 1 to 3 showed weak anti-microbial activities.

Keywords

marine fungus, *paraconiothyrium* sp., polyketide, ECD calculation, anti-microbial activities

Introduction

Marine-derived endophytic fungi refer to harmless parasitic fungi on the internal organs of plants and animals in a diverse marine environment without causing any adverse reactions. Host provided nutrients for endophytes, and endophytes produced bioactive substances giving the host advantage in survival competition. Symbionts coexist with symptomless fish, sponges, and soft corals that grow in a relatively harsh marine environment, characterized by high salinity, scarce nutrients, and high osmotic and hydraulic pressures, provided many environment-specific microorganisms, which could coevolve with their hosts by undergoing rapid and dynamic change of their genomes. Thus, marine-derived endophytic fungi are considered an important treasure trove of unique complex structural and diverse bioactive metabolites.

The genus *Paraconiothyrium* was discovered as a new genus by Verkley *et al* in 2004 and 27 species of *Paraconiothyrium* have been reported to date. However, limited secondary metabolites, including sesquiterpenes, diterpenes, polyketides and aromatic compounds, were isolated from the species in genus *Paraconiothyrium*. Most of them showed a wide range of biological activities, such as anti-inflammatory, antifungal, cytotoxic, phytotoxic, neuroprotective activities, and inhibiting the liver X receptor and HIV-1.

Previously, we mainly focused on the bioactive metabolites from marine-derived endophytic fungi. In our search for novel fungi-derived metabolites contributing to the development of drugs and the discovery of new pesticides, we systematically study the fermentation extract from strain *Paraconiothyrium* sp., which was originally isolated from the fiddler crab in Zhoushan island and was identified by its complete ITS gene sequences. The current study on the chemical constituents of strain *Paraconiothyrium* sp. Results in the isolation of one new naphthalenone, named paraconthone A (I), together with two known compounds 2 to 3 (Figure 1)12,13. Herein, we reported the isolation, structural identification and bio-evaluation of isolates.

Results and Discussion

The *Paraconiothyrium* sp. Extract was initially chromatographed on a DAD-HPLC column and then further purified by C18 reversed-phase HPLC to yield a new 3,4-dihydro-2H-naphthalen-1-one compound 1.

Paraconthone A (I) was obtained as a white solid, and its molecular formula was assigned to be C_{15}H_{18}O_{6} by HR-ESI-MS data ([M + Na]^{+} 317.0996; found, 317.0990) and interpretation of the 13C NMR data, indicating 7 degrees of unsaturation. The 1H NMR data (Table 1) displayed four clearly assigned protons, including an active hydrogen proton at δH 12.88 (s, 8-OH), a singlet aromatic proton at δH 6.67 (s, 8-OPH), a singlet aromatic proton at δH 6.67 (s, 5-OPH), and a singlet aromatic proton at δH 6.67 (s, 7-OPH).
H-5), and two methyl groups at δ_H 2.02 (s, 11-OAc), and 3.92 (s, 9-OMe). Meanwhile, a pair of characteristic triplet methylenes (δ_H 2.99, 2H, t, J = 7.0 Hz, H-10; δ_H 4.19, 2H, t, J = 7.0 Hz, H-11), a octet methylene (δ_H 2.90, 1H, ddd, J = 17.5, 9.5, 5.0 Hz, H-2b), a quartet methine (δ_H 4.86, 1H, dd, J = 5.5, 4.0) were observed combined HSQC spectra. The 15 carbon signals in the 13C NMR spectra (Table 1) were assigned with the help of the DEPT and HSQC experiment, indicating the presence of two methyl groups (δ_C 21.2 and 56.0), four methylenes (δ_C 22.0, 31.9, 34.9, and 63.0), one methine (δ_C 68.4) and seven quaternary carbons (δ_C 110.1, 112.6, 146.8, 162.8, 164.3, 171.3 and 202.3). Thus, compound 1 was assigned as a derivative of O-methylasparvenone. The planar structure of 1 was established by extensive HMBC (Figure 2) spectra. The key HMBC correlations from H2 to 2a and H2 to 2b to C-1, and C-8a, from H2 to 3 to C-4, C-4a, and from H-4 to C-4a, constructed a dicyclic benzocyclohexanone scaffold for compound 1. The further HMBC correlations from the olefinic alcohol group 8-OH to C-8, C-8a, and C-7 indicated the hydroxy group located at C-8. The correlations from H2 to 11 to 11-OAc, and from 6-MeO to C-6 assigned the attachment of OAc and MeO to C-11 and C-6, respectively, as well as the de-shielded shifts of C-11 (δ_C 63.0) and C-6 (δ_C 164.3). The correlation from H2 to 10 to C-7 indicated the attachment of 2-acetoxyethylene unite to C-7. Thus, the planar structure of 1 was established as 11-OAc- O-methylasparvenone.

Furthermore, the absolute configuration of 1 was defined by experimental and calculated electronic circular dichroism (ECD) data. The ECD spectra of 4R-1 and 4S-1 were calculated using the time-dependent density functional theory (TD-DFT) at the B3LYP/6 to 31+g (d, p) level. Therefore, 1 was deduced to have the 4R absolute configuration based on the well agreement between the calculated and measured ECD (Figure 3) and named paraconthone A.

Compounds 1 to 3 were assayed for two agricultural pathogenic bacteria (Xanthomonas oryzae pv. oryzae and Xanthomonas oryzae pv. oryzae) and four fish pathogens, Edwardsiella tarda, Vibrio anguillarum, Aeromonas hydrophilia, and Vibrio parahaemolyticus. Compounds 1 to 3 showed moderate inhibitory effects against the pathogenic bacteria at 30 μg/mL. Compounds 1

| NO. | δ_H (mult, J in Hz) | δ_C | Typea |
|-----|---------------------|-----|-------|
| 1   | 202.3 C             |     |       |
| 2   | 2.90 ddd (17.5, 7.0, 4.5) 2.62 ddd (17.5, 9.5, 5.0) | 34.9 | CH2   |
| 3   | 2.34 m 2.12 m | 31.9 | CH2   |
| 4   | 4.86 dd (5.5, 4.0) | 68.4 | CH    |
| 4a  | 112.6 C            |     |       |
| 5   | 6.67 s             | 100.5 | C     |
| 6   | 164.3 CH           |     |       |
| 7   | 112.6 C            |     |       |
| 8   | 162.8 C            |     |       |
| 8a  | 110.1 C            |     |       |
| 9   | 3.92 s             | 56.0 | CH3   |
| 10  | 2.99 t (7.0)       | 22.0 | CH2   |
| 11  | 4.19 t (7.0)       | 63.0 | CH2   |
| 8-OH| 12.88 s            |     |       |
| OAc | 2.02 s             | 171.3 | C     |

*a the carbon type was assigned by HSQC spectrum.

Figure 1. The structures of compounds 1 to 3.

Figure 2. HMBC correlations for compound 1.

Figure 3. Experimental and calculated ECD spectra (4R-1, blue dotted line) and (4S-1, red dotted line) of compound 1.
to 3 were also assayed for anti-inflammatory in acne-induced THP-1 cells and anti-tumor activities in A549, H460, MDA-MB-231 and BT549 cell lines. However, 1 to 3 showed no anti-inflammatory and cytotoxic activities at the concentration of 50 µM.

Experimental

General Experimental Procedures

NMR spectra were acquired on Bruker AVIII-600 NMR spectrometers using tetramethylsilane (TMS) as an internal standard. UV and ECD spectra were recorded on a Shimadzu UV-1800 spectrophotometer and chirascan circular dichroism spectrometers, respectively. The High-Resolution Electrospray Ionization Mass Spectrometry (HR-ESI-MS) was obtained with an Agilent 6529B Q-TOF instrument. The Optical rotation was measured on a JASCO P-1020 polarimeter in MeOH at 25 °C. Preparative high-performance liquid chromatography (Pre-HPLC) was performed by utilizing a Shimadzu LC-20 system equipped with a Shim-pack RP-C18 column (10 µm, 1.5 cm × 25 cm), which was recorded by a binary channel UV detector at 210 and 254 nm with a flow rate at 10 mL/min at room temperature. The Column chromatography (CC) was used with silica gel and ODS on a SepaBen Flash machine.

Fungal Material

The endophytic fungus was isolated from a sample of crab (Chirontes haematobrachis), which collected from the intertidal zone of Zhoushan, Zhejiang, China, in July 2018. The strain was identified as Paraconiothyrium sp. using ITS rDNA (accession number SUB10819600 ITS OI966906) sequence analysis by RuiDi (Shanghai, China) and its DNA sequence using BLAST was compared to the GenBank data. The fungal strain was deposited in the culture collection of the State Key Laboratory of Bioreactor Engineering laboratory, East China University of Science and Technology.

Fermentation, Extraction and Isolation

The fungus Paraconiothyrium sp. was incubated on potato dextrose agar (PDA) medium at 28 °C for approximately 2 to 3 days. Afterward, the agar plugs were cut into three pieces and transferred into 500 mL-Erlenmeyer flasks, containing 200 mL of potato dextrose broth (PDB). The flasks were cultured for two days at 28 °C on a rotary shaker at 180 rpm for inoculation. Then the seed cultures were added to the 20 × 1 L. flasks containing 200 mL malt extract medium (1000 mL deionized water contained 20 g malt extract, 20 g sucrose, and 1 g peptone), previously sterilized at 121 °C for 25 min. All flasks were incubated at 28 °C on a rotary shaker at 180 rpm for three days, and then they were transformed into the static culture for fourteen days at 28 °C.

The culture was filtered to give fermentation broth and mycelia, and the mycelia (170 g, wet weight) immersed in methanol (10 L) and then extracted with ultrasound, repeated three times to get a crude extract (5.0 g). The mycelia extract fractionated on silica gel with a stepped gradient elution with petroleum ether-EtOAc (10:1 to 1:1) to give four fractions (A–D) based on the results of TLC analysis. The fraction A (2.8 g) was sequentially fractionated by an ODS column eluting with MeOH-H2O (70% to 95%) to give six fractions (a–f). The subfraction b (130 mg) was sequentially loaded onto silica gel CC (dichloromethane-MeOH, 50:1) and preparative HPLC (MeCN-H2O, 55:45, 10.0 mL/min) to yield compound 1 (3.4 mg, tR 24.7 min), 2 (5.2 mg, tR 15.1 min) and 3 (5.2 mg, tR 32.6 min).

Spectroscopic Data

Paraconthone A (1): white solid; [α]D 25 D + 15.8° (c 0.10, MeOH); ECD (5 mg/L, MeOH) λmax (Δε) 209 (-3.49), 241 (-0.27), 249 (-0.44), 283 (0.84) nm; UV (MeOH) λmax (logε) 230 (3.46), 280 (4.00) nm; 1H and 13C NMR (CDCl3), see Table 1; positive HR-ESI-MS ([M + Na] 18) 317.0990, [M + Na] 19, (calcd. for C15H18NaO6 317.1099).

ECD Calculations

The detailed procedures are detailed in the Supporting Information.

Anti–Inflammatory Assays

The human monocytic cell line, THP-1 (Cell Bank of China Science Academy, Shanghai, China) and P. acnes (ATCC6919, Xiangfu biotech, Shanghai, China), were used for the anti-inflammatory assay. THP-1 cells were cultured in RPMI1640 medium with 10% fetal bovine serum (FBS, Gibco, NY, USA) in a humidified incubator (37 °C, 5% CO2). P. acnes bacteria were incubated in Cooked Meat Medium, containing cooked beef granules (Rishui biotechnology, Qingdao, China) in an anaerobic environment. The THP-1 cells were stimulated by the P. acnes, harvested at the exponential phase. The viability of THP-1 cells was evaluated by the MTT assay, specifically, seeding the THP-1 cells in 96-well plates at a density of 2 × 104 cells/well and treated with serially diluted compounds for 24 h (37 °C, 5% CO2). After that, adding 20 µL MTT regent (5 mg/mL, Genetimes Technology Inc., Shanghai, China) to each well and incubating the samples at 37 °C for 4 h. Removing the supernatant, the formazan crystals were fully solubilized in DMSO (150 µL), and the absorbance was measured at 570 nm and 630 nm wavelength. Tretinoin was used as a positive anti-inflammatory control. All the experiments were performed in three independent replicates.
Microorganisms Culture and Anti-Microbial Activities Assay

Minimum Inhibitory Concentration (MIC) assays were used to assess antimicrobial activities of all isolated compounds against two agricultural pathogenic bacteria (Xanthomonas oryzae pv. oryzae and Xanthomonas oryzae pv. oryzicola) and four fish pathogens, Edwardsiella tarda, Vibrio anguillarum, Aeromonas hydrophila, and Vibrio parahaemolyticus. Chloramphenicol was used as a positive antibacterial control and ketoconazole was used as a positive antifungal control. All the experiments were performed in three independent replicates.15

Cell Culture and Cell Viability Assay

Four kinds of cell lines including A549, H460, MDA-MB-231 and BT549 were cultured in RPMI-1640 medium (10% FBS, 1% P/S). Cells were incubated at 37 °C with 5% CO2 in a humidified atmosphere.16 5-Fu was used as a positive cytotoxicity control. All the experiments were performed in three independent replicates.

Ethical Approval

Ethical Approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

Supporting Information

Experimental procedures, computational details, biological activity determination procedure, and original spectra of compound I are available in Supporting Information section.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

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