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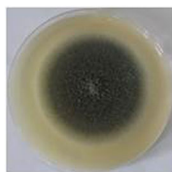
Three new unsaturated fatty acids from marine-derived fungus *Aspergillus* sp. SCAU150

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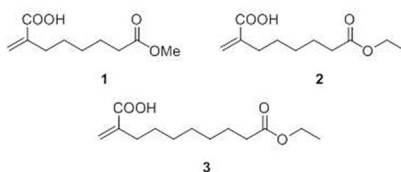
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ABSTRACT

Four unsaturated fatty acid derivatives including three new pantheric acids (**1–3**), together with three known polyketides (**5–7**), were isolated from a culture broth of the marine-derived fungus *Aspergillus* sp. SCAU150. Their complete structures were determined by NMR and HRESIMS data analyses. The antifungal activity of the isolated compounds above was evaluated and **2** was found to show moderated activity toward the phytopathogenic fungus *Fusarium solani* bio-80814 with an inhibition zone diameter of 6 mm under 5 µg/disc.



Aspergillus sp. SCAU150



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1. Introduction

Marine fungi are recognized as prolific sources of structurally unique secondary metabolites with promising biological activities (Jin et al. 2016; Carroll et al. 2020). Species of the genus *Aspergillus* are abundant producers of bioactive natural products for their utility in drug discovery, including antibacterial, antifungal, antiviral, antiparasitic, anti-cancer, and antidiabetic activities (Lee et al. 2013; Romsdahl and Wang 2019; Xu et al. 2020). Among them, the unsaturated fatty acids (UFAs) are a category of structures consisted of a long-chain hydrocarbon with the presence of at least one double covalent bond and ending in a carboxyl group (–COOH), which are of great interest in the

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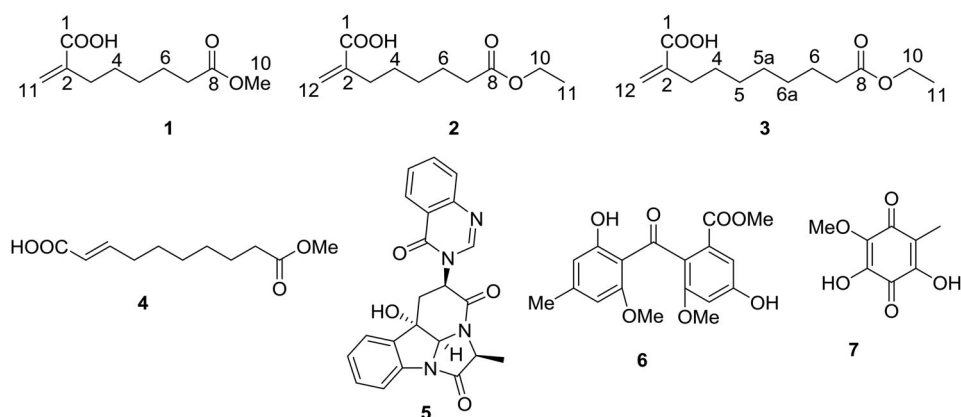


Figure 1. Chemical structures of compounds 1–7.

field of drug discovery because of their widespread bioactivities, such as antimalarial, antimicrobial (Calcul et al. 2013; Tang et al. 2019; Lu et al. 2019). During our ongoing search for structurally novel and biologically active compounds from marine-derived microorganisms (Nong et al. 2014; Nong et al. 2016), the secondary metabolites from a coral-associated fungus *Aspergillus* sp. SCAU150 were chemically investigated. As a result, three new unsaturated fatty acid derivatives pantheric acids D–F (**1–3**) featuring a terminal double bond, together with four known structures pantheric acid C (**4**) (Lee et al. 2019), chaetominine (**5**) (Jiao et al. 2006), monomethylsulochrin (**6**) (Guo et al. 2020), fumiquinone B (**7**) (Hayashi et al. 2007), were obtained from the fungal fermentation broth. The structures of these new compounds were elucidated by detailed analysis of NMR and HRESIMS data. And the antifungal activity of compounds **1–7** was also evaluated against a panel of six plant pathogenic fungal strains.

2. Results and discussion

The molecular formula of pantheric acid D (**1**) was assigned as $C_{10}H_{16}O_4$ by HRESIMS data with 3 degrees of unsaturation (Supplementary material Figure S9). Interpretation of the 1H NMR spectroscopic data of **1** (Supplementary material Table S1) displayed diagnostic signals for two shielded olefinic protons (δ_H 6.00, 5.52), one methoxy protons (δ_H 3.56). Thorough analysis of ^{13}C NMR spectroscopic data of **1** (Supplementary material Table S1) revealed resonances for two ester carbonyls (δ_C 173.4, 168.2), one sp^2 methylene (δ_C 124.1), one sp^2 none-protonated carbon (δ_C 141.3), five sp^3 methylene groups (δ_C 33.3, 31.3, 28.2, 27.8, 24.4) and one methoxy group (δ_C 51.2). These data provided a speculation that **1** was a fatty acid derivative. Further this was confirmed by observation of the HMBC spectrum of **1**, which showed correlations from H-3 to C-1/C-2/C-4/C-5/C-11, from H-7 to C-5/C-6/C-8, from Me-10 to C-8, and from H-11 to C-1/C-2/C-3 (Supplementary material Figure S1). The presence of a carboxylic acid group was determined at C-1 by the molecular formula, whose carbon appeared at δ_C 168.2. Based on above, the chemical structure of **1** was established as depicted (Figure 1). Additional analysis of the COSY spectrum of **1** showing cross-peaks between H-3 and both H-4 and H-11, and between H-6 and H-7, also supported the

above assignment (Supplementary material Figure S1). Considering the structure of compound **1** was an analogue of pantheric acid C (**4**) (Lee et al. 2019), it was given a trivial name as pantheric acid D.

The molecular formula of pantheric acid E (**2**) was assigned as $C_{11}H_{18}O_4$ by HRESIMS data with 3 degrees of unsaturation (Supplementary material Figure S16). The 1H NMR spectroscopic data of **2** (Supplementary material Table S1) exhibited obvious signals for two shielded protons (δ_H 6.01, 5.55), one oxygenated methylene quartet (δ_H 4.06), one methyl triplet (δ_H 1.18). The ^{13}C NMR spectroscopic data of **2** (Supplementary material Table S1) showed signals for two ester carbonyls (δ_C 172.9, 168.1), one sp^2 methylene (δ_C 124.2), one sp^2 none-protonated carbon (δ_C 141.0), six sp^3 methylene groups including one oxygenated methylene (δ_C 59.6), and one methyl (δ_C 14.1). The 1H and ^{13}C NMR spectroscopic data of **2** were greatly similar to those of **1** with the only obvious difference of the absence of one methoxy group (δ_C 51.2 in compound **1**) and the additional appearance of one oxygenated methylene and one methyl group (δ_C 59.6, 14.1) in **2**. Careful analysis of the 2D NMR spectra revealed that **2** was an analogue of **1**, with a methoxy group attached to C-8 instead of an oxygenated ethyl group, which was supported by the COSY spectrum showing the presence of a spin system of H₂-10/Me-11 and the HMBC spectrum exhibiting correlations from Me-11 to C-10, from H₂-10 to C-8 (Supplementary material Figure S1). Therefore, the structure of **2** was confidently defined as depicted in Figure 1, and given a trivial name as pantheric acid E.

The molecular formula of pantheric acid F (**3**) was assigned as $C_{13}H_{22}O_4$ by HRESIMS data with 3 degrees of unsaturation (Supplementary material Figure S23). The 1H and ^{13}C NMR spectroscopic data of **3** (Supplementary material Table S1) was very similar to **2** with the only obvious difference of the addition of two deshielded methylenes (δ_C 28.3, 28.4). Comprehensive analysis of HMBC spectrum disclosed correlations from H-3 to C-1/C-2/C-4/C-5/C-12, from H-4 to C-2/C-3/C-5/5a, from H-7 to C-6/C-6a/C-8, from H₂-10 to C-8/C-11 and from Me-11 to C-10 (Supplementary material Figure S1), while the COSY spectrum revealed spin systems of H-3 and H-4/H-12, H-6 and H-6a/H-7, H-10 and H-11 (Supplementary material Figure S1). So the structure of **3** was readily elucidated and given a trivial name of pantheric acid F.

Considering the new compounds above may be artifacts owing to the uses of methanol, ethyl acetate and acetone as elution solvents of column chromatography during the separation process, the initial extract was used to perform a test of LC-HRESIMS experiment. The result indicated the presence of compounds **1–3** in the original crude extract (Supplementary material Figure S24). To the best of our knowledge, the methyl/ethyl ester formation for fatty acids were also naturally occur like iedomycin A (Mondol et al. 2011), fusarisolin E (Niu et al. 2019), methylester-3-*O*- α -L-rhamnopyranoside and (–)-9-hydroxyhexylitaconic acid (Antia et al. 2011), hexadecanoic acid ethyl ester and 9,12-octadecadienoic acid ethyl ester (Marante et al. 2012), (10*Z*,13*Z*)-ethyl nonadeca-10,13-dienoate and (9*Z*,12*Z*)-ethyl nonadeca-9,12-dienoate (Feng et al. 2015). And it is reported that the biosynthesis of these fatty acids methyl/ethyl ester is involved in two different enzymatic mechanisms, esterification or alcoholysis (Saerens et al. 2006; Herrera-Valencia et al. 2012).

3. Experimental

3.1. General experimental procedures

IR spectra were recorded on a FT-IRNICOLET spectrophotometer. UV spectra were collected on a Beckman DU 640 spectrophotometer. NMR spectra were recorded on a Bruker AV spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). TMS was used as a reference. HRESIMS spectra were measured on an amaZon SL ion trap mass spectrometer and MaXis quadrupole-time-of-flight mass spectrometer (Bruker). Semi-preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C18 column (9.4×250 mm, $5 \mu\text{m}$). Silica gel (Qing Dao Hai Yang Chemical Group Co.; 100–200, 200–300 mesh) were used for open column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin-layer chromatography (TLC).

3.2. Biological material

Fungal strain SCAU150 was isolated from the stony coral *Acropora digitifera* from Subi Reef in the South China Sea ($9^{\circ}39'\text{N}$, $112^{\circ}59'\text{E}$). The strain was identified as an *Aspergillus* based on BLAST analysis of fungal ribosomal DNA sequence of internal transcribed spacers (GenBank accession MT597427), which was 100% similarity with that of *Aspergillus fumigatus* strain MEBP0074. Strain SCAU150 was deposited in the Joint Laboratory of Guangdong Province and Hong Kong Region on Marine Bioresource Conservation and Exploitation, College of Marine Sciences, South China Agricultural University.

3.3. Fermentation and extraction

Fungal strain *Aspergillus sp.* SCAU150 was cultured in 125 replicate 1000 mL Erlenmeyer flasks each containing 400 mL of potato dextrose broth (PDB) supplemented with 3% sea salt, under static incubation at 28°C for 28 days. At the end of the fermentation period, the culture broth was separated from the mycelium by filtration. The mycelium was extracted with acetone, while the culture filtrate was extracted with EtOAc solvent. A combined organic extract was afforded. It was noted that the fermentation and isolation work was performed as previous literatures (Liu et al. 2015; Zhao et al. 2015).

3.4. Isolation and purification

The total extract (20 g) was subjected to silica gel CC with a gradient elution petroleum ether (PE)/ethyl acetate (EtOAc) from 100:0 to 50:50 (v/v) and $\text{CHCl}_3/\text{MeOH}$ from 90:10 to 0:100 (v/v), affording six fractions (Fr.1 ~ 6) by TLC analysis. Fraction Fr.2 (2 g) was fractionated by CC (200–300 mesh) with a PE/EtOAc gradient solvent system (from 10% to 80%) to obtain four sub-fractions (Fr.2-1 ~ Fr.2-4). Fraction Fr.2-1 was purified by repeated semi-preparative reversed-phase (SP-RP) HPLC (Agilent Eclipse XDB-C18 column, 250×9.4 mm, $5 \mu\text{m}$; 2 mL/min flow rate) with MeOH/ H_2O (70: 30) to

obtain **6** (5 mg, $t_R = 8$ min). Compound **5** (4 mg, $t_R = 45$ min) was acquired by repeated SP-RP HPLC with MeOH/H₂O (70: 30) from fraction F2-2. Fraction Fr.3 (5 g) was fractionated by CC eluted with CHCl₃/acetone solvent system (from 10% to 100%), collecting six sub-fractions (Fr.3-1~ Fr.3-6). Fraction Fr.3-1 was prepared by repeated SP-RP-HPLC to yield **7** (4 mg, $t_R = 8$ min) with MeOH/H₂O (70: 30) elution solvent system. Fraction Fr.3-2 was separated by repeated SP-RP-HPLC to generate **1** (4 mg, $t_R = 7$ min) with MeOH/H₂O (70: 30) as eluent. Fraction Fr.3-3 was isolated by repeated SP-RP-HPLC to attain **2** (4 mg, $t_R = 5$ min) using MeOH/H₂O mobile phase with a volume ratio at 70: 30. Fraction Fr.3-4 was purified by repeated SP-RP-HPLC to attain **4** (5 mg, $t_R = 6$ min) with MeOH/H₂O (70: 30) as eluent. Fraction Fr.3-5 was isolated by repeated SP-RP-HPLC to gain **3** (6 mg, $t_R = 8$ min) with MeOH/H₂O (70: 30) elution solvent system.

3.4.1. Pantheric acid D (1)

Pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 205 (3.30); IR (MeOH) ν_{max} : 2937, 2862, 1693, 1625, 1172 cm⁻¹; ¹H and ¹³C NMR data, [Supplementary material Table S1](#); HRESIMS m/z 199.0977 [M-H]⁻ (calcd for C₁₀H₁₆O₄, 199.0976).

3.4.2. Pantheric acid E (2)

Pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 205 (3.61); IR (MeOH) ν_{max} : 2931, 2864, 1699, 1627, 1186 cm⁻¹; ¹H and ¹³C NMR data, [Supplementary material Table S1](#); HRESIMS m/z 213.1136 [M-H]⁻ (calcd for C₁₁H₁₇O₄, 213.1132).

3.4.3. Pantheric acid F (3)

Pale yellow oil; UV (MeOH) λ_{max} (log ϵ) 205 (3.17); IR (MeOH) ν_{max} : 2929, 2856, 1695, 1627, 1180 cm⁻¹; ¹H and ¹³C NMR data, [Supplementary material Table S1](#); HRESIMS m/z 241.1453 [M-H]⁻ (calcd for C₁₃H₂₁O₄, 241.1445).

3.5. Antifungal assay

Hitherto numerous unsaturated fatty acids and related derivatives were reported to show a wide range of antimicrobial activities (Alves et al. 2020). In this work, compounds **1–7** were tested antifungal activity against six plant phytopathogenic fungi of *Colletotrichum asianum* HNM 408, *Colletotrichum acutatum* HNMRC 178, *Fusarium oxysporum* HNM 1003, *Pyricularia oryzae* HNM 1003, *Fusarium solani* bio-80814 and *Fusarium moniliforme* bio-52799 by disc diffusion method as described previously (Huang et al. 2020). The final result revealed compound **2** exhibited moderated activity toward the pathogenic fungus *Fusarium solani* bio-80814 with an inhibition zone diameter of 6 mm under 5 μ g/disc, while carbendazim (Sigma-Aldrich) was used as a positive control showing an inhibition zone diameter of 20 mm under the same sample sizes ([Supplementary material Figure S2](#)). Even though there have been several reports regarding the mode of action of unsaturated fatty acids, the precise mechanism for the antimicrobial activity remains unclear. Recently, it was suggested that the antimicrobial activity of unsaturated fatty acids by targeting different cellular functions

including protein synthesis and involving in morphogenesis, adhesion and biofilm formation, as well as membrane perturbations (Bhattacharyya et al. 2020).

4. Conclusion

Three new unsaturated fatty acid derivatives (**1–3**) and four known compounds (**4–7**) were isolated from the marine-derived fungus *Aspergillus* sp. SCAU150. Compounds **1–3** have been characterized by NMR and HRESIMS data. In addition, Compounds **1–7** were evaluated antifungal activity and **2** showed moderated activity toward the pathogenic fungus *Fusarium solani* bio-80814.

Disclosure statement

No potential conflict of interest was reported by the authors.

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