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

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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.

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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, anticancer, 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
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 \(^1\)H NMR spectroscopic data of 1 (Supplementary material Table S1) displayed diagnostic signals for two shielded olefinic protons (\(\delta_H 6.00, 5.52\)), one methoxy protons (\(\delta_H 3.56\)). Thorough analysis of \(^{13}\)C NMR spectroscopic data of 1 (Supplementary material Table S1) revealed resonances for two ester carbonyls (\(\delta_C 173.4, 168.2\)), one \(sp^2\) methylene (\(\delta_C 124.1\)), one \(sp^2\) none-protonated carbon (\(\delta_C 141.3\)), five \(sp^3\) methylene groups (\(\delta_C 33.3, 31.3, 28.2, 27.8, 24.4\)) and one methoxy group (\(\delta_C 51.2\)). 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 \(\delta_C 168.2\). 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 $^1$H NMR spectroscopic data of 2 (Supplementary material Table S1) exhibited obvious signals for two shielded protons ($\delta_H$ 6.01, 5.55), one oxygenated methylene quartet ($\delta_H$ 4.06), one methyl triplet ($\delta_H$ 1.18). The $^{13}$C NMR spectroscopic data of 2 (Supplementary material Table S1) showed signals for two ester carbonyls ($\delta_C$ 172.9, 168.1), one sp$^2$ methylene ($\delta_C$ 124.2), one sp$^2$ none-protonated carbon ($\delta_C$ 141.0), six sp$^3$ methylene groups including one oxygenated methylene ($\delta_C$ 59.6), and one methyl ($\delta_C$ 14.1). The $^1$H 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 ($\delta_C$ 51.2 in compound 1) and the additional appearance of one oxygenated methylene and one methyl group ($\delta_C$ 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 H$_2$-10/Me-11 and the HMBC spectrum exhibiting correlations from Me-11 to C-10, from H$_2$-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 $^1$H 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 ($\delta_C$ 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 H$_2$-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 ieodomycin A (Mondol et al. 2011), fusarisolin E (Niu et al. 2019), methylester-3-O-\(\alpha\)-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), (10Z,13Z)-ethyl nonadeca-10,13-dienoate and (9Z,12Z)-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-IR NICOLET spectrophotometer. UV spectra were collected on a Beckman DU 640 spectrophotometer. NMR spectra were recorded on a Bruker AV spectrometer (400 MHz for $^1$H 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 μm). 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°39′N, 112°59′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 CHCl$_3$/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, S-5 μm; 2 mL/min flow rate) with MeOH/H$_2$O (70: 30) to
obtain 6 (5 mg, $t_R = 8 \text{ min}$). Compound 5 (4 mg, $t_R = 45 \text{ min}$) was acquired by repeated SP-RP HPLC with MeOH/H$_2$O (70: 30) from fraction F2-2. Fraction Fr.3 (5 g) was fractionated by CC eluted with CHCl$_3$/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, $t_R = 8 \text{ min}$) with MeOH/H$_2$O (70: 30) elution solvent system. Fraction Fr.3-2 was separated by repeated SP-RP-HPLC to generate 1 (4 mg, $t_R = 7 \text{ min}$) with MeOH/H$_2$O (70: 30) as eluent. Fraction Fr.3-3 was isolated by repeated SP-RP-HPLC to attain 2 (4 mg, $t_R = 5 \text{ min}$) using MeOH/H$_2$O 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, $t_R = 6 \text{ min}$) with MeOH/H$_2$O (70: 30) as eluent. Fraction Fr.3-5 was isolated by repeated SP-RP-HPLC to gain 3 (6 mg, $t_R = 8 \text{ min}$) with MeOH/H$_2$O (70: 30) elution solvent system.

3.4.1. Pantheric acid D (1)
Pale yellow oil; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 205 (3.30); IR (MeOH) $\nu_{\text{max}}$: 2937, 2862, 1693, 1625, 1172 cm$^{-1}$; $^1$H and $^{13}$C NMR data, Supplementary material Table S1; HRESIMS $m/z$ 199.0977 [M-H]$^-$ (calcd for C$_{10}$H$_{16}$O$_4$, 199.0976).

3.4.2. Pantheric acid E (2)
Pale yellow oil; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 205 (3.61); IR (MeOH) $\nu_{\text{max}}$: 2931, 2864, 1699, 1627, 1186 cm$^{-1}$; $^1$H and $^{13}$C NMR data, Supplementary material Table S1; HRESIMS $m/z$ 213.1136 [M-H]$^-$ (calcd for C$_{11}$H$_{17}$O$_4$, 213.1132).

3.4.3. Pantheric acid F (3)
Pale yellow oil; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 205 (3.17); IR (MeOH) $\nu_{\text{max}}$: 2929, 2856, 1695, 1627, 1180 cm$^{-1}$; $^1$H and $^{13}$C NMR data, Supplementary material Table S1; HRESIMS $m/z$ 241.1453 [M-H]$^-$ (calcd for C$_{13}$H$_{21}$O$_4$, 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 μg/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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References

Alves E, Dias M, Lopes D, Almeida A, Domingues MR, Rey F. 2020. Antimicrobial lipids from plants and marine organisms: an overview of the current state-of-the-art and future prospects. Antibiotics. 9(8):441–528.

Antia BS, Aree T, Kasettrathat C, Wiyakrutta S, Ekpa OD, Ekpe UJ, Mahidol C, Ruchirawat S, Kittakoop P. 2011. Itaconic acid derivatives and diketopiperazine from the marine-derived fungus Aspergillus aculeatus CRI322-03. Phytochemistry. 72(8):816–820.

Bhattacharyya A, Sinha M, Singh H, Patel RS, Ghosh S, Sardana K, Ghosh S, Sengupta S. 2020. Mechanistic insight into the antifungal effects of a fatty acid derivative against drug-resistant fungal infections. Front Microbiol. 11:2116.

Calcul L, Waterman C, Ma WS, Lebar MD, Harter C, Mutka T, Morton L, Maignan P, Olphen AV, Kyle DE, et al. 2013. Screening mangrove endophytic fungi for antimalarial natural products. Mar Drugs. 11(12):5036–5050.

Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR. 2020. Marine natural products. Nat Prod Rep. 37(2):175–223.

Feng MT, Yu XQ, Yang P, Yang H, Lin K, Mao SC. 2015. Two new antifungal polyunsaturated fatty acid ethyl esters from the red alga. Chem Nat Compd. 51(3):418–422.

Guo C, Lin XP, Liao SR, Yang B, Zhou XF, Yang XW, Tian XP, Wang JF, Liu YH. 2020. Two new aromatic polyketides from a deep-sea fungus Penicillium sp. SCSIO 06720. Nat Prod Res. 34(9):1197–1205.

Hayashi A, Fujioka S, Nukina M, Kawano T, Shimada A, Kimura Y. 2007. Fumiquinones A and B, nematicidal quinones produced by Aspergillus fumigatus. Biosci Biotechnol Biochem. 71(7):1697–1702.

Herrera-Valencia VA, Us-Vázquez RA, Larqué-Saavedra FA, Barahona-Pérez LF. 2012. Naturally occurring fatty acid methyl esters and ethyl esters in the green microalga Chlamydomonas reinhardtii. Ann Microbiol. 62(2):865–870.
Huang ZB, Xiao XJ, Huang ZH, Xu L, Zhang XY, Tang RY. 2020. Selective C-H dithiocarbamation of arenes and antifungal activity evaluation. Org Biomol Chem. 18(7):1369–1376.

Jiao RH, Xu S, Liu JY, Ge HM, Ding H, Xu C, Zhu HL, Tan RX. 2006. Chaetominine, a cytotoxic alkaloid produced by endophytic Chaetomium sp. IFB-E015. Org Lett. 8(25):5709–5712.

Jin LM, Quan CS, Hou XY, Fan SD. 2016. Potential pharmacological resources: natural bioactive compounds from marine-derived fungi. Mar Drugs. 14(4):76–100.

Lee SR, Yi SA, Nam KH, Ryoo R, Lee J, Kim KH. 2019. Pantheric acids A-C from a poisonous mushroom, Amanita pantherina, promote lipid accumulation in adipocytes. J Nat Prod. 82(12):3489–3493.

Lee YM, Kim MJ, Li HY, Zhang P, Bao BQ, Lee KJ, Jung JH. 2013. Marine-derived Aspergillus species as a source of bioactive secondary metabolites. Mar Biotechnol. 15(5):499–519.

Liu Y, Li XM, Meng LH, Jiang WL, Xu GM, Huang CG, Wang BG. 2015. Bisthiodiketopiperazines and acorane sesquiterpenes produced by the marine-derived fungus Penicillium admetzioides AS-53 on different culture media. J Nat Prod. 78(6):1294–1299.

Lu YF, Chen YN, Wu YL, Hao HL, Liang WJ, Liu J, Huang RM. 2019. Marine unsaturated fatty acids: structures, bioactivities, biosynthesis and benefits. RSC Adv. 9(61):35312–35327.

Marante FJT, Mioso R, Barrera JB, González JEG, Rodríguez JJS, Laguna IHB. 2012. Structural characterization and metabolite profiling of the facultative marine fungus Paecilomyces variotii. Ann Microbiol. 62(4):1601–1607.

Mondol MAM, Kim JH, Lee MA, Tareq FS, Lee HS, Lee YJ, Shin HJ. 2011. leodomycins A-D, antimicrobial fatty acids from a marine bacillus sp. J Nat Prod. 74(7):1606–1612.

Nong XH, Wang YF, Zhang XY, Zhou MP, Xu XY, Qi SH. 2014. Territrem and butyrolactone derivatives from a marine-derived fungus Aspergillus terreus. Mar Drugs. 12(12):6113–6124.

Nong XH, Zhang XY, Xu XY, Wang J, Qi SH. 2016. Nahuic acids B-E, polyhydroxy polyketides from the marine-derived Streptomyces sp. SCGAA 0027. J Nat Prod. 79(1):141–148.

Ni SW, Tang XX, Fan ZW, Xia JM, Xie CL, Yang XW. 2019. Fusarisolins A–E, polyketides from the marine-derived fungus Fusarium solani H918. Mar. Drugs. 17(2):125–136.

Romstdahl J, Wang CCC. 2019. Recent advances in the genome mining of Aspergillus secondary metabolites (covering 2012–2018). Med Chem Commun. 10(6):840–866.

Saerens SMG, Verstrepen KJ, Van Laere SDM, Voet ARD, Van Dijck P, Delvaux FR, Thevelein JM. 2006. The Saccharomyces cerevisiae EHT1 and EEB1 genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. J Biol Chem. 281(7):4446–4456.

Tang XX, Yan X, Fu WH, Yi LQ, Tang BW, Yu LB, Fang MJ, Wu Z, Qiu YK. 2019. New ß-lactone with tea pathogenic fungus inhibitory effect from marine-derived fungus MCCC3A00957. J Agric Food Chem. 67(10):2877–2885.

Xu K, Yuan XL, Li C, Li XD. 2020. Recent discovery of heterocyclic alkaloids from marine-derived Aspergillus species. Mar Drugs. 18(1):54–75.

Zhao Q, Chen GD, Feng XL, Yu Y, He RR, Li XX, Huang Y, Zhou WX, Guo LD, Zheng YZ, et al. 2015. Nodulisporivoridins A-H, bioactive viridins from Nodulisporium sp. J Nat Prod. 78(6):1221–1230.