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Two new unsaturated fatty acids from the mangrove rhizosphere soil-derived fungus *Penicillium javanicum* HK1-22

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ABSTRACT

Two new unsaturated fatty acids, 6*R*,8*R*-dihydroxy-9*Z*,12*Z*-octadecadienoic acid (**1**) and methyl-6*R*,8*R*-dihydroxy-9*Z*,12*Z*-octadecadienoate (**2**), and two known 9*Z*,12*Z*-octadecadienoic acid analogues (**3**, **4**) together with a known sesquiterpenoid (**5**) were isolated from the mangrove rhizosphere soil-derived fungus *Penicillium javanicum* HK1-22. An acetonide derivative (**1a**) from **1** was also prepared. The relative configuration of **1** was determined by analysis of the 1D and 2D NOE spectra of **1a**. The absolute configuration of **1** was assigned on the basis of biogenetic considerations. The antifungal activity of the high yield compound **5** was evaluated against four strains of crop pathogens and it showed significant antifungal activities against all the tested strains.

1. Introduction

Marine-derived microorganisms have been regarded as an important bioresources of structurally diverse natural products with pronounced biological activities. Specially, mangrove-associated microorganisms inhabiting in tropical and subtropical intertidal estuarine zones have the biosynthetic potential to produce a wide range of novel secondary metabolites [1–3]. Fatty acids are common products in all biological systems, including animals, plants, and fungi. For fungi, the metabolism of fatty acid is associated with important processes of the fungal life cycle, such as spore formation. It is worth noting that mangrove rhizosphere soil-derived fungi are one of the major groups of biological organisms that provide significant number of unsaturated fatty acids with pharmacological properties [4]. Undoubtedly, unsaturated fatty acids are of great interest in the field of drug discovery, because these compounds have widespread bioactivities. In the context of ongoing projects aimed at the description of the chemical diversity present in mangrove-derived fungi from the South China Sea, lots of bioactive compounds including azaphilones [5,6], indole alkaloid [7] and naphtho-γ-pyrones [8] were obtained. For the purpose of searching for novel active compounds from mangrove rhizosphere soil-derived fungi, a strain of *Penicillium javanicum* HK1-22 was further chemically investigated. As a result, two new unsaturated fatty acids (**1**, **2**) and two known analogues (**3**, **4**) together with a known sesquiterpenoid (**5**) (Fig. 1) was isolated from the fungal cultures. Furthermore, the antibacterial and antifungal activities of these compounds were also evaluated in this paper.

2. Results and discussion

The fungus *P. javanicum* HK1-22 was isolated from mangrove rhizosphere soil collected from Dongzhaigang mangrove natural reserve in Hainan Island, China. The identification of HK1-22 was based on its morphological traits and a molecular protocol by amplification and sequencing of the DNA sequences of the ITS region of the rRNA gene. The phylogenetic tree based on the ITS rRNA gene sequences of the closely related species was constructed by neighbor joining tree method and illustrated in Fig. 2. HK1-22 was closely related to *P. javanicum* CBS 129771 (MH877078.1), sequence had been submitted to NCBI GenBank with accession number of MK790264. Chemically investigated the

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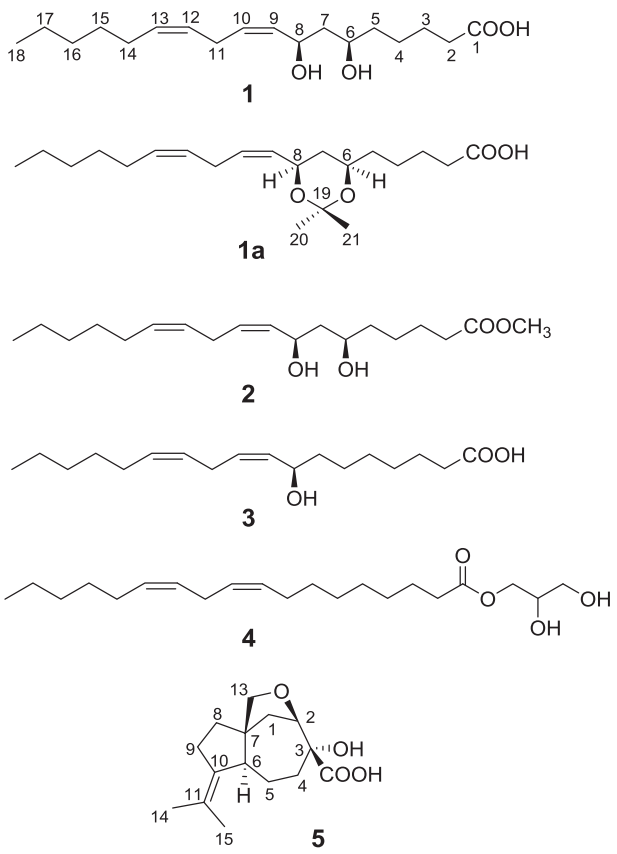

Fig. 1. Structures of compounds **1–5** and **1a**.

fungus *P. javanicum* HK1-23 led to the isolation of four unsaturated fatty acids (**1–4**) and a known sesquiterpenoid (**5**) by using chromatographic techniques including column chromatography and semi-preparative HPLC.

Compound **1** was isolated as a brown oil. Its molecular formula was determined as $C_{18}H_{32}O_4$ (three degrees of unsaturation) by HRESIMS. This molecular formula was also corroborated by ^{1}H and ^{13}C NMR spectroscopic data (Table 1). The ^{1}H NMR spectrum of **1** (Table 1) showed four olefinic or aromatic signals at δ_H 5.52, 5.45, 5.41, 5.30, a methyl signal at δ_H 0.89 (d, J = 6.6 Hz), two oxymethine signals at δ_H 3.96, 4.82, and ten methylene signals. The ^{13}C NMR spectrum revealed that **1** contained a carbonyl (δ_C 178.5), four olefinic or aromatic carbon atoms, as well as thirteen aliphatic series carbon atoms. These spectroscopic features suggested that **1** is very similar to 8*R*-hydroxy-9*Z*,12*Z*-octadecadienoic acid (**3**), a fatty acid previously obtained from the culture of *Laetisaria arvalis* [9]. The significant difference between these two compounds was that one more oxymethine signal at δ_H 3.96 was present in **1** compared to those in **3**. The geometry of the two double bonds at C-9 and C-12 in **1** was *Z* configuration according to the coupling constants in the ^{1}H NMR spectrum (J = 10.7, 7.8 Hz and J = 10.7, 7.2 Hz, respectively). Further detailed analysis of the COSY, HSQC and HMBC (Fig. 3) experiments unambiguously assigned the planar structure of **1**.

In order to determine the relative configuration of **1**, we obtained the acetonide derivative of **1** (**1a**). Then the relative configuration of **1** was determined by analysis of the 1D and 2D NOE (Fig. 4) spectra of **1a**. In the NOESY spectrum of **1a**, the correlations of H-6/CH_3-20 and H-8/CH_3-20 suggested that H-6 and H-8 were located at the same side of the acetonide ring. In the selective 1D NOE experiment, the irradiation of H-6 and H-8 resulted in the enhancement of CH_3-20 (δ_H 1.39), which further confirmed H-6 and H-8 should be on the same face of the molecule of **1a**. On the other hand, linoleic acid is a very common metabolite in fungi. A literature research revealed that linoleic acid is catalyzed by a (8*R*)-dioxygenase to form 8*R*-hydroxy-octadecadienoic acid via the formation of a hydroperoxide in many strains of fungi [10–12]. From a biosynthesis point of view, it is reasonable that compounds **1–3** were proposed as respective 8*R*-configuration derivatives from linoleic acid. Combined with the relative configuration of the acetonide derivative (**1a**), compound **1** was determined as 6*R*,8*R*-dihydroxy-9*Z*,12*Z*-octadecadienoic acid.

Compound **2** was also isolated as a brown oil. Its molecular formula was determined as $C_{19}H_{34}O_4$ by HRESIMS. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The significant difference between them was that a methoxyl group signal (1-OCH_3, δ_H 3.67 and δ_C 51.5) was present in **2** compared to compound **1**. In the HMBC spectrum of **2**, the correlations from 1-OCH_3 to C-1 displayed that the 1-OCH_3 was connected to the carbonyl. The planar structure of **2** was independently assigned by analysis of its HSQC, COSY and HMBC data. Compound **2** was the methyl ester product of **1** isolated from the same fungal strain simultaneously. Therefore, compound **2** could be assigned as methyl-6*R*,8*R*-dihydroxy-9*Z*,12*Z*-octadecadienoate from a biosynthetic standpoint. In addition, it seems that **2** may arise from **1** during the extraction or purification processes with MeOH. However, when **1** was dissolved in MeOH with freshly prepared silica gel and stirred at room temperature for 1 week, **2** was not detected in the solution. Therefore, **2** is proposed as a natural product.

The known compounds **3–5** were determined to be 8*R*-hydroxy-9*Z*,12*Z*-octadecadienoic acid (**3**) [9], 1-*O*-(9*Z*, 12*Z*-octadecadienoyl) glycerol (**4**) [13] and aspterric acid (**5**) [14] based on their NMR, ESI-MS, and by comparison with data previously reported in the literature.

All isolated metabolites (**1–5**) were evaluated for their antibacterial activity against a panel of pathogenic bacteria. However, none of these compounds were proved to be active. The high yield compound **5** was evaluated for its antifungal activity by the mycelium linear growth rate method against four crop pathogenic fungi including *Rhizoctonia solania*, *R. cerealis*, *Gaeumannomyces graminis* and *Alternaria alternate* at 50, 10 and 2 μg/mL. The results (Fig. 5, Table S1) showed that compound **5** exhibited significant antifungal activities against the four tested crop pathogens with the inhibition rates ranging from 78.67% to 100% at 50 μg/mL and from 12.67% to 43% at 10 μg/mL, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on an Anton Paar MCP300 automatic polarimeter. UV spectra were obtained on a Beckman DU 640 spectrophotometer. IR spectra were recorded on a Cary 610/670 spectrometer using KBr pellets. NMR spectra were acquired using an AVANCE 600 NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C), using TMS as internal standard. HRESIMS spectra were obtained from a maXis spectrometer. Semi-preparative HPLC was performed on a HITACHI system using a semi-preparative C_{18} (Kromasil, 5 μm, 10 × 250 mm) column coupled with a 2400 UV detector. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh), and Sephadex LH-20 (Amersham Biosciences) were used for 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. Microbial methods

3.2.1. Sample collection

The mangrove rhizosphere soil sample was collected from mangrove rhizosphere soil (GPS coordinates, 19°57′19.2″N, 110°35′12.4″E; depth of sample collection, 20–30 cm) from Dongzhaigang mangrove natural reserve in Hainan Island, China, in September 2015. The sample was stored in sterilized polythene hand bags and put on ice, then transported to the laboratory and processed immediately for isolation and cultivation of fungi. Details of the soil sample were exposed as follows,

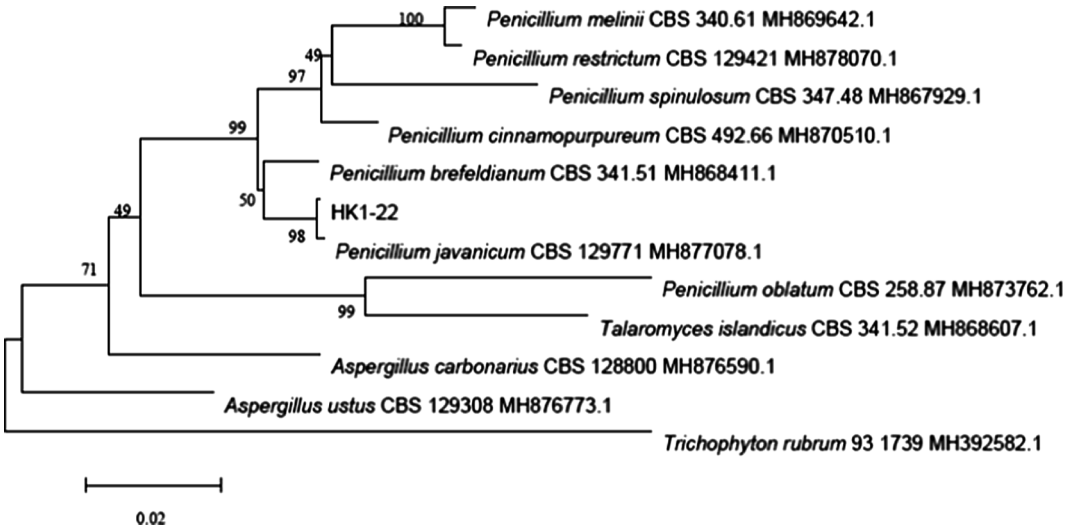

Fig. 2. Phylogenetic tree of ITS rRNA sequences of closely related *P. javanicum* (HK1-22). Reference sequences were downloaded from NCBI with the accession numbers indicated in parentheses. Distances and clustering was performed by MEGA 4.1. Bootstrap values based on 1000 replications are listed at the branching point.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for **1** and **2** ($CDCl_3$).

Position	**1**		**2**	
	δ_C, type	δ_H, mult. (*J* in Hz)	δ_C, type	δ_H, mult. (*J* in Hz)
1	178.5, C	–	174.2, C	–
2	33.9, CH_2	2.36, t, 7.2	34.0, CH_2	2.33, t, 7.8
3	24.6, CH_2	1.65, m	24.8, CH_2	1.68, m
4	25.1, CH_2	1.48, m	25.2, CH_2	1.49, m
5	36.9, CH_2	1.55, m	37.1, CH_2	1.57, m
6	68.8, CH	3.96, m	68.9, CH	3.96, m
7	43.0, CH_2	1.69, m	42.9, CH_2	1.73, m
8	65.6, CH	4.82, dt, 7.8, 3.6	65.7, CH	4.82, dt, 7.8, 3.6
9	132.0, CH	5.52, dtt, 10.7, 7.8, 1.4	132.2, CH	5.52, dtt, 10.7, 7.8, 1.4
10	130.1, CH	5.45, ddt, 10.7, 7.8, 1.4	130.2, CH	5.47, ddt, 10.7, 7.8, 1.4
11	26.0, CH_2	2.85, m	26.0, CH_2	2.82, m
12	126.9, CH	5.30, dt, 10.7, 7.2	126.8, CH	5.32, dt, 10.7, 7.2
13	131.1, CH	5.41, dtt, 10.7, 7.2, 1.5	131.1, CH	5.41, dtt, 10.7, 7.2, 1.5
14	27.2, CH_2	2.04, q, 7.2	27.3, CH_2	2.04, q, 7.2
15	29.3, CH_2	1.36, m	29.2, CH_2	1.39, m
16	31.5, CH_2	1.30, m	31.5, CH_2	1.34, m
17	22.6, CH_2	1.28, m	22.6, CH_2	1.30, m
18	14.1, CH_3	0.89, d, 6.6	14.1, CH_3	0.89, d, 7.2
1-OCH_3	–	–	51.5, CH_3	3.67, s

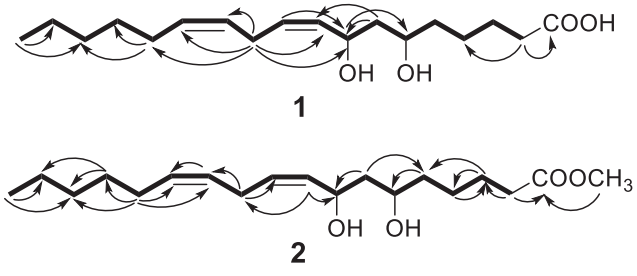

Fig. 3. Key HMBC (⟶) and $^{1}H-^{1}H$ COSY(▬) correlations of **1** and **2**.

pH, 5.2; salinity, 7.8‰ and organic matter, 2.11%.

3.2.2. Isolation

The fungus *Penicillium javanicum* HK1-22 was isolated by the serial dilution method (1:10, 1:100, and 1:1000) using potato dextrose agar

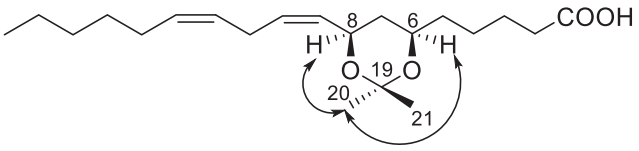

Fig. 4. Key NOE correlations for **1a**.

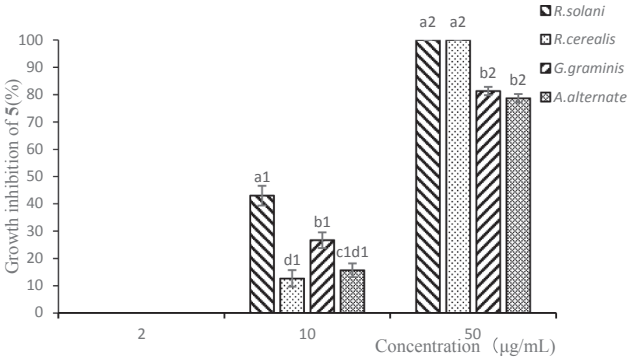

Fig. 5. Antifungal activities of compound **5.**

medium (30 g of natural sea salt, 200 g of potato, 20 g of dextrose, 1000 mL of H_2O) incorporated with ampicillin (25 mg/L) and streptomycin sulfate (25 mg/L). The strain was deposited at the Marine Science & Technology Institute, College of Environmental Science & Engineering, Yangzhou University, Yangzhou, PR China.

3.2.3. Identification

The fungus was identified according to its morphological traits and a molecular protocol by amplification and sequencing of the DNA of the ITS region of the rRNA gene as described previously [15]. The DNA quality was checked and amplified using ITS1-(5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4-(5′-TCC TCC GCT TAT TGA TAT GC-3′) primers. The sequence data have been submitted to GenBank with accession number MK790264, and the fungus was identified as a *P. javanicum*, whose 593 base pair ITS sequence had 98% sequence identity to that of *P. javanicum* CBS 129771 (MH877078.1).

3.2.4. Fermentation

The fungal strain *P. javanicum* HK1-22 was cultivated statically in

potato dextrose broth medium (30 g of natural sea salt, 200 g of potato, 20 g of dextrose, 1000 mL of H_2O) in 1 L Erlenmeyer flasks (50 flasks) for 30 days with light at room temperature.

3.3. Chemical extraction, isolation and characterisation

The fermentation broth was filtered to separate the broth from the mycelia. Then the broth was extracted three times with an equal volume of EtOAc, and the mycelia were extracted three times with MeOH. The organic extracts were combined and concentrated under vacuum to afford a total extract (40.0 g), which was subjected to silica gel column chromatography (CC) using a step gradient elution with EtOAc-petroleum ether (0–100%) and then with MeOH-EtOAc (0–100%) for separation into five fractions (Fr.1–Fr.5). Fr.4 was subjected to Sephadex LH-20 and further purified on semi-preparative HPLC (75% MeOH/H_2O) to give **3** (3.0 mg) and **4** (4.0 mg). And Fr.4.3 was recrystallization to give **5** (70.0 mg). Fr.5 was purified by silica gel CC and further purified on semi-preparative HPLC (70% MeOH/H_2O) to obtain **1** (30.0 mg) and **2** (8.0 mg).

6*R*,8*R*-Dihydroxy-9*Z*,12*Z*-octadecadienoic acid (**1**): brow oil; $[\alpha]_D^{20}$ + 29 (*c*, 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 205 (1.1) nm; IR (KBr) ν_{max} 3369, 2938, 2864, 1708, 1054, 1033, 1018 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; HRESIMS *m/z* 335.2192 $[M + Na]^+$ (calcd for $C_{18}H_{32}NaO_4$, 335.2193).

Methyl-6*R*,8*R*-dihydroxy-9*Z*,12*Z*-octadecadienoate (**2**): brow oil; $[\alpha]_D^{20}$ + 25 (*c*, 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 218 (0.15), 203 (0.7) nm; IR (KBr) ν_{max} 3368, 2940, 2865, 1722, 1664, 1454 1053, 1033, 1018 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; HRESIMS *m/z* 349.2355 $[M + Na]^+$ (calcd for $C_{19}H_{34}NaO_4$, 349.2349).

3.4. Preparation of the acetonide derivative 1a

Compound **1** (5.0 mg) was treated with 2,2-dimethoxypropane (1.5 mL) and p-toluenesulfonic acid (0.5 mg) in acetone (3.0 mL) at room temperature for 30 min. Saturated aqueous $NaHCO_3$ (5 mL) was then added, and the reaction mixture was extracted with EtOAc (5 mL × 3). The organic solvents were removed under a vacuum, and the crude mixture was subjected to semipreparative HPLC to obtain **1a** (4.2 mg) as a brown oil.

((4*R*,6*R*)-6-((1*Z*,4*Z*)-Deca-1,4-dien-1-yl)-2,2-Dimethyl-1,3-dioxan-4-yl)pentanoic acid **(1a):** brown oil; ^1H NMR ($CDCl_3$, 600 MHz) δ_H 5.47 (1H, dtt, *J* = 10.7, 7.2, 1.4 Hz, H-9), 5.41 (1H, ddt, *J* = 10.7, 7.2, 1.4 Hz, H-10), 5.40 (1H, dtt, *J* = 10.7, 7.2, 1.5 Hz, H-13), 5.31 (1H, dt, *J* = 10.7, 7.2 Hz, H-12), 4.66 (1H, dt, *J* = 8.4, 3.6 Hz, H-8), 3.79 (1H, m, H-6), 2.86 (2H, m, H-11), 2.03 (2H, m, H-2), 1.51 (2H, m, H-4), 1.39 (3H, s, H-20), 1.35 (3H, s, H-21), 1.70 (2H, m, H-7), 1.32 (6H, m, H-15, 16, 17), 0.88 (3H, d, *J* = 6.6 Hz, H-18); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C 178.5 (C, C-1), 131.2 (CH, C-9), 131.0 (CH, C-13), 130.1 (CH, C-12), 126.9 (CH, C-10), 100.2 (C, C-19), 66.5 (CH, C-6), 63.0 (CH, C-8), 38.9 (CH_2, C-7), 36.9 (CH_2, C-5), 35.8 (CH_2, C-2), 31.5 (CH_2, C-16), 29.3 (CH_2, C-15), 27.2 (CH_2, C-14), 26.2 (CH_2, C-11), 25.8 (CH_3, C-20), 25.5 (CH_3, C-21), 25.2 (CH_2, C-4), 24.8 (CH_2, C-3), 22.6 (CH_2, C-17), 14.1 (CH_3, C-18); HRESIMS *m/z* 375.2510 $[M + Na]^+$ (calcd for $C_{21}H_{36}NaO_4$, 375.2506).

3.5. Biological assays

3.5.1. Antibacterial activity

Seven bacterial strains, including Gram-positive methicillin-resistant *Staphylococcus aureus* (ATCC 43300, 33591), -susceptible *S. aureus* (ATCC 25923, 29213), *Enterococcus faecalis* (ATCC 51299), *E. faecium* (ATCC 35667), and Gram-negative *Escherichia coli* (ATCC 25922), were used for antibacterial assay. The specific antibacterial assay was carried out as described previously [16].

3.5.2. Antifungal activity

Four crop pathogenic fungal strains, including *Rhizoctonia Solani*, *R. cerealis*, *Gaeumannomyces graminis*, *Alternaria alternate*, were used for antifungal assay. The fungal species are courtesy of College of Horticulture and Plant Conservation, Yangzhou University. The specific antifungal assay was carried out as described previously [17].

4. Conclusions

In summary, four unsaturated fatty acids (**1**–**4**) and a sesquiterpenoid (**5**) were isolated from the mangrove rhizosphere soil-derived fungus *P. javanicum* HK1-22. The planar structures of the new compounds (**1**, **2**) were determined by detailed analysis of the 1D and 2D spectroscopic data. The relative configuration of **1** was assigned by analysis of the 1D and 2D NOE spectra of the acetonide derivative (**1a**) prepared from **1**. Aspterric acid (**5**) displayed significant antifungal activities against the crop pathogens including *R. Solani*, *R. cerealis*, *G. graminis*, and *A. alternate* and could be interesting lead compound for further development of novel fungicides.

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Appendix A. Supplementary material

NMR and HRESIMS spectra of **1**, **2** and **1a** and map of the location of the sampling site. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103331.

References

[1] J.W. Blunt, A.R. Carroll, B.R. Copp, et al., Marine natural products, Nat. Prod. Rep. 35 (2018) 8–53.
[2] J. Xu, Bioactive natural products derived from mangrove-associated microbes, RSC Adv. 5 (2014) 841–892.
[3] E. Ancheeva, G. Daletos, P. Proksch, Lead compounds from mangrove-associated microorganisms, Mar. Drugs 16 (2018) 319–350.
[4] X.X. Tang, X. Yan, W.H. Fu, et al., New *β*-lactone with tea pathogenic fungus inhibitory effect from marine-derived fungus MCCC3A00957, J. Agric. Food. Chem. 67 (2019) 2877–2885.
[5] M. Chen, N.X. Shen, Z.Q. Chen, et al., Penicilones A-D, anti-MRSA azaphilones from the marine-derived fungus *Penicillium janthinellum* HK1-6, J. Nat. Prod. 80 (2017) 1081–1086.
[6] M. Chen, Y.Y. Zheng, Z.Q. Chen, et al., NaBr-induced production of brominated azaphilones and related tricyclic polyketides by the marine-derived fungus *Penicillium janthinellum* HK1-6, J. Nat. Prod. 82 (2019) 368–374.
[7] Y.Y. Zheng, N.X. Shen, Z.Y. Liang, et al., Paraherquamide J, a new prenylated indole alkaloid from the marine-derived fungus *Penicillium janthinellum* HK1-6, Nat. Prod. Rep. (2019), https://doi.org/10.1080/14786419.2018.1534105.
[8] Y.Y. Zheng, Z.Y. Liang, N.X. Shen, et al., New naphtho-γ-pyrones isolated from marine-derived fungus *Penicillium* sp. HK1-22 and their antimicrobial activities, Mar. Drugs 17 (2019) 322.
[9] M.W. Wadman, G.V. Zadelhoff, M. Hamberg, et al., Conversion of linoleic acid into novel oxylipins by the mushroom *Agaricus bisporus*, Lipids 40 (2015) 1163–1170.
[10] U. Garscha, F. Jernerén, D. Chung, et al., Identification of dioxygenases required for *Aspergillus* development. Studies of products, stereochemistry, and the reaction

mechanism, J. Biol. Chem. 282 (2007) 34707–34718.
[11] I.D. Brodowsky, M. Hamberg, E.H. Oliw, A linoleic acid (8*R*)-dioxygenase and hydroperoxide isomerase of the fungus *Gaeumannomyces graminis*. Biosynthesis of (8*R*)-hydroxylinoleic acid and (7*S*,8*S*)-dihydroxylinoleic acid from (8*R*)-hydroperoxylinoleic acid, J. Biol. Chem. 267 (1992) 14738–14745.
[12] I.D. Brodowsky, E.H. Oliw, Biosynthesis of 8*R*-hydroperoxylinoleic acid by the fungus *Laetisaria arvalis*, BBA 1168 (1993) 68–72.
[13] D.G. Kim, M.G. Kang, S.S. Hong, et al., Antiinflammatory effects of functionally active compounds isolated from aged black garlic, Phytother. Res. 31 (2016) 53–61.
[14] Y. Tsuda, M. Kaneda, A. Tada, et al., Aspterric acid, a new sesquiterpenoid of the carotane group, a metabolite from *Aspergillus terreus* IFO-6123. X-ray crystal and molecular structure of its p-bromobenzoate, J. Chem. Soc., Chem. Commun. 4 (1978) 160–161.
[15] C.J. Zheng, C.L. Shao, Z.Y. Guo, et al., Bioactive hydroanthraquinones and anthraquinone dimers from a soft coral-derived *Alternaria* sp. fungus, J. Nat. Prod. 75 (2012) 189–197.
[16] C.G. Pierce, P. Uppuluri, A.R. Tristan, et al., A simple and reproducible 96 well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing, Nat. Protoc. 3 (2008) 1494–1500.
[17] R. Yang, Z.F. Gao, J.Y. Zhao, et al., New class of 2-aryl-6-chloro-3,4-dihydroisoquinolinium salts as potential antifungal agents for plant protection: synthesis, bioactivity and structure–activity relationships, J. Agric. Food. Chem. 63 (2015) 1906–1914.