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BRIEF COMMUNICATION

Two new secondary metabolites from a mangrove-derived fungus *Cladosporium* sp. JS1-2

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Abstract

One new pentenoic acid derivative, named 1,1′-dioxine-2,2′-dipropionic acid (**1**) and a new natural product, named 2-methylacetate-3,5,6-trimethylpyrazine (**2**), along with six known compounds (**3**–**8**), were obtained from the *Cladosporium* sp. JS1-2, an endophytic fungus isolated from the mangrove *Ceriops tagal* collected in South China Sea. Their structures were elucidated by detailed analysis of comprehensive spectroscopic data, and the structure of **1** was further determined by X-ray diffraction analyses. ^{13}C NMR chemical shifts of structure **2** was further determined by GIAO based ^{13}C NMR chemical shifts calculations. Compounds **1**–**4** and **6** showed growth inhibition activities against newly hatched larvae of *Helicoverpa armigera Hubner* with the IC_{50} values ranging from 100 to 150 $\mu g\ ml^{-1}$. Compounds **1**, **2**, **4**, **6** and **7** showed moderate antibacterial activities against *Staphylococcus aureus* with the MIC values of 25.0, 12.5, 6.25, 1.25, and 6.25 $\mu g\ ml^{-1}$, respectively.

During the last decade, marine-derived fungi have proven to be a prolific source of structurally novel and biologically active natural compounds, which gained considerable attention [1, 2]. Especially the fungus isolated from mangrove could produce structurally novel and bioactive compounds, such as antiosteoporotic citrofulvicin [3], anti-inflammatory chrysogenester [4], antibacterial brocapyrrozin A [5], and antivirus simpterpenoid A [6]. In our search for new bioactive natural products from mangrove-derived fungi in the South China Sea, we have found many bioactive compounds, including cytotoxic indole diterpenes, chlorinated xanthones, anthraquinone derivatives, dihydroisocoumarins and isocoumarins [7–11]. In our ongoing search for new bioactive compounds from mangrove-derived fungus, an endophytic fungus *Cladosporium* sp. JS1-2 obtained from the mangrove *Ceriops tagal*, was selected for further research because its EtOAc extract showed insecticidal activity against newly hatched larvae of *Helicoverpa armigera Hubner*. One new pentenoic acid derivative 1,1'-dioxine-2,2'-dipropionic acid (**1**) and a new natural product 2-acetate-3,5,6-trimethylpyrazine (**2**) and six known compounds (**3**–**8**) were isolated from the EtOAc extract of the fungus (Fig. 1). In this report, we described the isolation, structure elucidation, antimicrobial and insecticidal activities of all compounds.

The fungus *Cladosporium* sp. JS1-2 was isolated from the mangrove plant *C. tagal*, collected from Dongzhaigang of Hainan Province in China in July, 2016. The strain was deposited in the Key Lab of Tropical Medicinal Resource Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, P.R. China. The fungus was identified according to its morphological characteristics and a molecular biological protocol by 18S rRNA amplification and sequencing of the ITS region. The sequence data have been submitted to GenBank, with an accession number MK234874, and the fungal strain was identified as *Cladosporium* sp.

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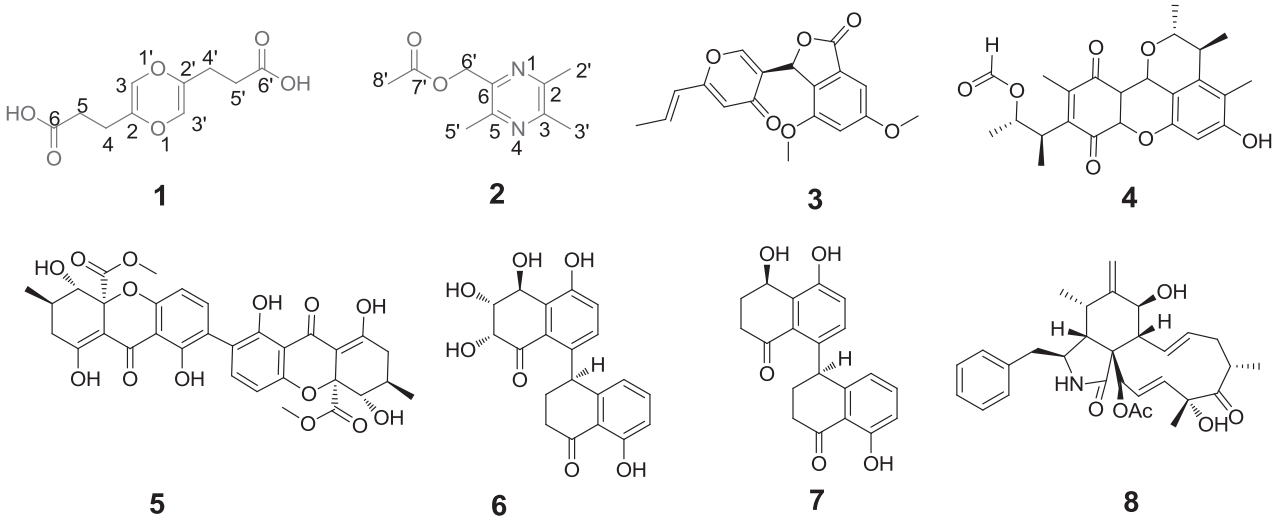

Fig. 1 The structure of compounds **1**–**8**

The fungus *Cladosporium* sp. JS1-2 was cultured in 200 ml of potato dextrose broth at 30 °C on a rotary shaker (120 rpm) for 5 days to prepare the seed culture. Large-scale fermentation was carried out in 200-Erlenmeyer flasks (1000 ml), each containing potato dextrose broth (8 g), and purified water (200 ml), which were soaked overnight before autoclaving at 121 °C for 20 min. These Erlenmeyer flasks were added to 3 ml seed broth after cooling to room temperature, and maintained at room temperature for 30 days in stationary phase.

The fungal cultures was extracted with EtOAc (3 × 10 L, 24 h each), which were filtered through cheesecloth. The extracts were concentrated in vacuo to yield an oily residue (25.3 g), which was subjected to silica gel CC (petroleum ether, EtOAc v/v, gradient 100:0–0:100) to generate six fractions (Fr. A−Fr. E). Fr. C was chromatographed on a silica gel column by stepwise-gradient elution using petroleum ether/ethyl acetate from 100: 0 to 0: 100. The fractions were further purified with preparative HPLC with an isocratic solvent system of 0.1% formic acid in 30% acetonitrile-water at a flow rate of 2 ml min^{-1} to obtain compounds **1** (7 mg) and **4** (3.1 mg). Fr. D was further purified by Sephadex LH20 column ($CHCl_3$/MeOH v/v, 1: 1) and eluted with preparative HPLC with an isocratic solvent system of 0.1% formic acid in 40% acetonitrile-water at a flow rate of 2 ml min^{-1} to obtain compounds **2** (3.4 mg) and **6** (3.7 mg). Like the above method, we obtained **3** (4.0 mg) and **7** (4.4 mg) from Fr. B, and acquired **5** (3.3 mg) and **8** (3.9 mg) from Fr. E.

Compound **1** was obtained as colorless crystals and had a molecular formula of $C_{10}H_{12}O_6$ as determined by the HR-ESI-MS at *m/z* 251.0529 $[M + Na]^+$ (calcd. for 251.0526), indicating five degrees of unsaturation. The ^{13}C NMR and DEPT 135 spectra data (Table 1) exhibited 5 carbon signals,

Table 1 ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of compounds **1** and **2**

Position	1[a]		2[b]	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1				
2	153.0, C		148.4, C	
3	143.3, CH	8.45, s	150.8, C	
4	29.1, CH_2	2.95, t (7.6)		
5	32.2, CH_2	2.67, t (7.6)	144.6, C	
6	173.7, C		148.5, C	
1'				
2'	153.0, C		20.0, CH3	2.44, s
3'	143.3, CH	8.45, s	21.2, CH3	2.42, s
4′	29.1, CH_2	2.95, t (7.6)		
5'	32.2, CH_2	2.67, t (7.6)	21.0, CH3	2.43, s
6′	173.7, C		64.4, CH2	5.12, s
7′			170.1, C	
8′			20.5, CH_3	2.06, s

[a]DMSO-d_6

[b]CD_3OD

including one carbonyl carbon (δ_C 173.3), one double bond (δ_C 153.0 and 143.3) and two methylene carbons (δ_C 32.2 and 29.1). Since one carbonyl group and one double bond accounted for two out of five degrees of unsaturation, implying that **1** was a dimer, and the remaining one degree of unsaturation were assumed for the presence of one ring system in **1**. The ^1H NMR spectrum of **1** (Table 1) in DMSO-d_6 showed one downfield proton signal at δ_H 8.45 (1 H, s), two methylene proton signals at δ_H 2.95 (2 H, t, $J = 7.6$ Hz), and 2.67 (2 H, t, $J = 7.6$ Hz). The ^1H-^1H COSY correlation of H-4 and H-5, together with the HMBC

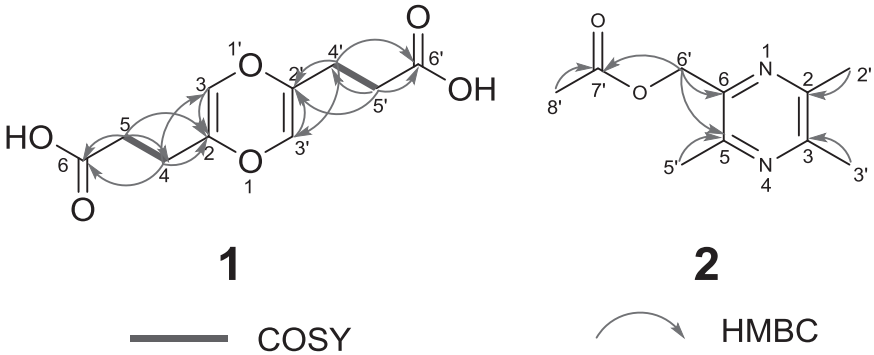

Fig. 2 ^{1}H-^{1}H COSY (in blue lines) and HMBC (in red lines) correlations of **1**–**2**

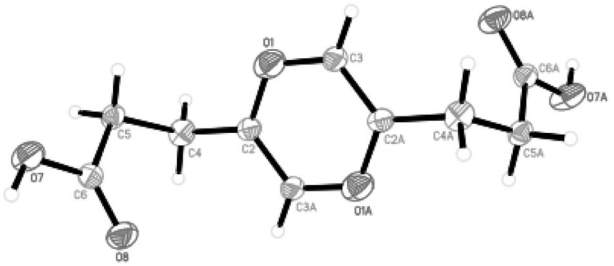

Fig. 3 X-ray structureof Compound **1**

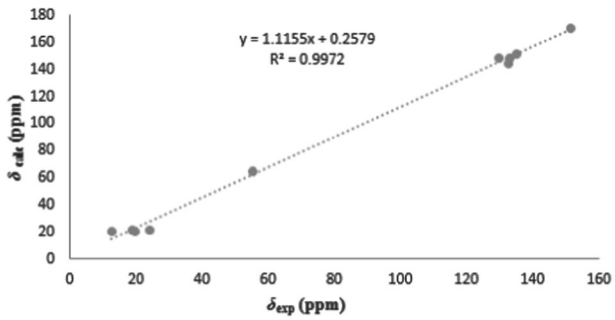

Fig. 4 Regression analysis of experimental versus calculated 13C NMR chemical shifts of compound **2** [at the B3LYP/6-31G(d)// B3LYP/6-31G(d, p)level]

correlations (Fig. 2) from H-3 to C-2; from H-4 to C-2, C-3, C-5, and C-6; from H-5 to C-2, C-4, and C-6, indicated that **1** comprised two (*Z*)- 5-hydroxypent-4-enoic acid units and it was a symmetrical dimer. The structure of **1** was also determined by X-ray diffraction analyses (Fig. 3). Hence, compound **1** was determined as 1,1'-dioxine-2,2'-dipropionic acid.

Compound **2** was isolated as yellow powder and gave a HR-ESI-MS ion peak at *m/z* 195.1133 $[M + H]^+$ (calcd for 195.1128), corresponding to a molecular formula of $C_{10}H_{14}N_2O_2$ with five degrees of unsaturation. The ^{13}C NMR and DEPT 135 spectra (Table 1) data exhibited 10 carbon signals, including one ester carbonyl carbon (δ_C 170.1), two double bonds (δ_C 150.8, 148.5, 148.4, and 144.6), one oxygenated methylene carbon (δ_C 64.4), and four methyl carbons (δ_C 21.2, 21.0, 20.5, and 20.0). The ^{1}H NMR spectrum of **2** (Table 1) showed one methylene proton signal at δ_H 5.12 (2 H, s, H-6'), four methyl protons at δ_H 2.44 (3 H, s, H-2'), 2.43 (3 H, s, H-5'), 2.42 (3 H, s, H-3'), and 2.06 (3 H, s, H-8'). The HMBC correlations (Fig. 2) from H-8' to C-7'; from H-6' to C-5, C-6, and C-7'; from H-2' to C-2; from H-3' to C-3; from H-5' to C-5, primary ascertained the structure of compound **2**.

According to the NMR spectra of compound **2**, we were uncertain the location of C-2 (δ_C 148.4) and C-3 (δ_C 150.8). To further verify the structure, a calculation of the ^{13}C NMR chemical shifts of structure **2** at the B3LY/6-31 G (d)// B3LYP/6-31 G (d, p) level with the PCM model in DMSO was obtained [12], and the calculated chemical shifts agreed well with the experimental data (Fig. 4), of which the Fig. 4 showed a correlation coefficient (R^2) of 0.9972, indicating that the calculation of the ^{13}C NMR chemical shifts of structure **2** was suitable. Finally **2** was determined as 2,3,5-trimethyl-6-methylacetate-pyrazine. Interestingly, compound **2** was firstly reported as a synthetic intermediate for the synthesis of piperlongumine-ligustrazine hybrids [13] and **2** was isolated from a natural source for the first time.

The structures of known compounds **3**–**8** were identified by comparison of their ^{1}H/^{13}C NMR spectra with those in the literature as vermistatin (**3**) [14], citrinin H1 (**4**) [15], secalonic acid D (**5**) [16], ladosporol E (**6**) [17], cladosporol C (**7**) [18], and cytochalasin D (**8**) [19].

All compounds were evaluated for their antibacterial activities against five terrestrial pathogenic bacteria, including *S. aureus* (ATCC 27154), *Staphylococcus albus* (ATCC 8799), *B. cereus* (ATCC 11778), *Escherichia coli* (ATCC 25922), and *Micrococcus luteus* (ATCC 10240) by the microplate assay method [20]. The result (Table 2) showed that **1**, **2**, **4**, **6** and **7** showed antibacterial activities against *S. aureus* with the MIC values of 25.0, 12.5, 6.25, 1.56 and 6.25 μg ml^{-1}, respectively. Ciprofloxacin was used as positive control with the MIC value of 0.39 μg ml^{-1}.

Compounds **1**–**8** were also evaluated for growth inhibition activity against newly hatched larvae of *H. armigera Hubner* [21]. Compounds **1**–**4** and **6** showed growth inhibition activities against newly hatched larvae of *H. armigera Hubner* with the IC_{50} values of 150, 100, 150, 100 and

Table 2 Antibacterial activity for compounds 1–8

Compound	MIC (μg ml^{-1})				
	S. aureus	*E. coli*	*S. albus*	*B. cereus*	*M. luteus*
1	25.0	25.0	>25.0	12.5	>25.0
2	12.5	>25.0	>25.0	>25.0	>25.0
3	25.0	>25.0	>25.0	25	>25.0
4	6.25	12.5	>25.0	12.5	>25.0
5	>25	>25.0	>25.0	>25.0	>25.0
6	1.56	>25.0	>25.0	>25.0	12.5
7	6.25	>25.0	>25.0	>25.0	12.5
8	25.0	>25.0	>25.0	>25.0	>25.0
Ciprofloxacin[a]	0.39	0.19	0.39	0.39	0.39

[a]Ciprofloxacin was used as a positive control

150 μg ml^{-1}, respectively. Azadirachtin was used as positive control with the IC_{50} value of 25 μg ml^{-1}.

Compound **1**: colorless crystals. mp. 108–110 °C; IR (KBr) ν_{max} 3536, 1750, 1315 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HR-ESI-MS *m/z* 251.0529 $[M + Na]^+$ (calcd. for $C_{10}H_{12}O_6Na^+$, 251.0526).

Compound **2**: yellow powder. IR (KBr) ν_{max} 1598, 1445, 873 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HR-ESI-MS *m/z* 195.1133 $[M + H]^+$ (calcd. for $C_{10}H_{15}N_2O_2^+$, 195.1128).

Crystal data for **1**: $C_{10}H_{12}O_6$, Mr = 600.60, monoclinic, $a = 5.6567$ (3) Å, $b = 13.2069$ (5) Å, $c = 8.0189$ (5) Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 523.64$ (5) Å^3, space group $P2_12_12_1$, $Z = 2$, $D_x = 1.447$ mg/mm^3, μ (Cu Kα) = 0.816 mm^{-1}, and F (000) = 240. Independent reflections: 924 ($R_{int} = 0.0619$). The final R_1 values were 0.0566, $wR_2 = 0.1185$ ($I > 2\sigma$ (I)).

In summary, we isolated and characterized one new pentenoic acid derivative **1** and a new natural product **2**, along with other six known compounds (**3**-**8**) from the fungus *Cladosporium* sp. JS1-2. Compounds **1** and **2** showed insecticidal activities against *H. armigera Hubner* and antimicrobial activities against *S. aureus*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Carroll AR, et al. Marine natural products. Nat Prod Rep. 2019;36:122–73.
2. Rateb ME, et al. Secondary metabolites of fungi from marine habitats. Nat Prod Rep 2011;28:290–344.
3. Chen Y, et al. Citrofulvicin, an antiosteoporotic polyketide from *Penicillium velutinum*. Org Lett. 2018;20:3741–4.
4. Liu S, et al. An anti-inflammatory PPAR-γ agonist from the jellyfish-derived fungus *Penicillium chrysogenum* J08NF-4. J Nat Prod. 2018;81:356–63.
5. Meng LH, et al. Antimicrobial alkaloids produced by the mangrove endophyte *Penicillium brocae* MA-231 using the OSMAC approach. Rsc Adv. 2017;7:55026–33.
6. Li HL, et al. Simpterpenoid A, a meroterpenoid with a highly functionalized cyclohexadiene moiety featuring *gem*-propane-1,2-dione and methylformate groups, from the mangrove-derived *Penicillium simplicissimum* Ma-332. Org Lett 2018;20:1465–8.
7. Zheng CJ, et al. Penicilindoles A-C, cytotoxic indole diterpenes from the mangrove-derived fungus *Eupenicillium* sp. HJ002. J Nat Prod. 2018;84:1045–9.
8. He KY, et al. New chlorinated xanthone and anthraquinone produced by a mangrove-derived fungus *Penicillium citrinum* HL-5126. J Antibiot. 2017;70:823–7.
9. Zhou XM, et al. Bioactive anthraquinone derivatives from the mangrove-derived fungus *Stemphylium* sp. 33231. J Nat Prod. 2014;77:2021–8.
10. Huang GL, et al. Dihydroisocoumarins from the mangrove-derived fungus *Penicillium citrinum*. Mar Drugs. 2016;14:1771–8.
11. Mei RQ, et al. Two new isocoumarins isolated from a mangrove-derived fungus *Penicillium citrinum* HL-5126. Chinese J Org Chem. 2019; https://doi.org/10.6023/cjoc201812008.
12. Pierens GK. ^{1}H and ^{13}C NMR scaling factors for the calculation of chemical shifts in commonly used solvents using density functional theory. J Comput Chem. 2014;35:1388–94.
13. Klein B, et al. Pyrazines. II. the rearrangement of pyrazine-*N*-oxides. J Org Chem. 1961;26:126–31.
14. Liu YY, et al. Vermistatin derivatives with α-Glucosidase inhibitory activity from the mangrove endophytic fungus *Penicillium* sp. HN29-3B1. Planta Med. 2014;80:912–7.
15. Ngan NTT, et al. Anti-inflammatory effects of secondary metabolites isolated from the marine-derived fungal strain *Penicillium* sp. SF-5629[J]. Arch Pharmacal Res. 2017;40:328–37.
16. Liu F, et al. The bioactive metabolites of the mangrove endophytic fungus *Talaromyces* sp. ZH-154 isolated from *Kandelia candel* (L.) Druce. Planta Med. 2010;76:185–9.
17. Zhang ZZ, et al. Clindanones A and B and cladosporols F and G, polyketides from the deep-sea derived fungus *Cladosporium cladosporioides* HDN14-342†. RSC Adv. 2016;6:76498–504.
18. Li HL, et al. Characterization of cladosporols from the marine algal-derived endophytic fungus *Cladosporium cladosporioides* EN-399 and configurational revision of the previously reported cladosporol derivatives. J Org Chem. 2017;82:9946–54.
19. Xu H, Fang WS, Chen XG, He WY, Cheng KD. Cytochalasin D from *Hypocrella Bambusae*. J Asian Nat Prod Res. 2001;3:151–5.
20. Pierce CG, 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. 2008;3:1494–500.
21. Guo ZK, et al. Metabolites with insecticidal activity from *Aspergillus fumigatus* JRJ111048 isolated from mangrove plant *Acrostichum specioum* endemic to Hainan Island. Mar Drugs. 2017;15:381–7.