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Two new secondary metabolites from a mangrove-derived fungus *Cladosporium* sp. JJM22

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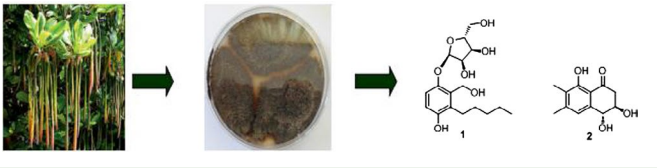
ABSTRACT

Two new compounds (**1** and **2**), together with six known compounds (**3–8**), were obtained from the *Cladosporium* sp. JJM22, an endophytic fungus isolated from the stem bark of the mangrove plant *Ceriops tagal* collected in South China Sea. Their structures were elucidated on the basis of detailed spectroscopic analysis. The absolute configurations of **1** and **2** were confirmed by the comparison of optical rotation and the CD data with those of known compounds. The inhibitory activities of the isolated compounds against six terrestrial pathogenic bacteria and human cervical carcinoma Hela cell line were evaluated. Compound **3** exhibited a broad spectrum of antibacterial activities.



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Ceriops tagal; endophytic fungus; ribofuranose phenol; naphthalene derivative; antibacterial activity

1. Introduction

It is acknowledged that there is a wealthy source of structurally unique and biologically potent secondary metabolites from marine-derived fungi (Li et al., 2017; Vinale et al. 2017). Recently, the mangrove-derived fungus has become an important source for the discovery of new natural compounds. In our search for new bioactive metabolites from mangrove-derived fungi, some bioactive compounds have been found (Zhou et al. 2014; Huang et al. 2016; He et al. 2017). Recently, the fungus *Cladosporium* sp. JJM22 isolated from the stem bark of *C. tagal* captured our attention because the EtOAc extract of rice medium of the fungus exhibited antimicrobial activity against *Staphylococcus aureus*. Moreover, a literature

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survey revealed that a series of biologically active compounds have been reported from the marine fungus *Cladosporium* sp. including isoindolone derivatives and alkaloids with antiviral activities (Peng et al. 2013), cladospolides E and F with potent lipid-lowering activity in HepG2 hepatocytes (Zhu et al. 2015), 3*α*-hydroxy-7-ene-6,20-dione with antiviral activities against respiratory syncytial virus (RSV) (Yu et al. 2017). In this study, chemical investigation on the EtOAc extract of the fermentation broth led to the identification of one new ribofuranose phenol derivative, 4-*O*-*α*-*D*-ribofuranose-3-hydroxymethyl-2-pentylphenol (**1**), one new naphthalene derivative, (-)-*trans*-(3*R*,4*R*)-3,4,8-trihydroxy-6,7-dimethyl-3,4- dihydronaphthalen-1(2*H*)-one (**2**), together with six known compounds, (3*S*)-3,8-dihydroxy-6,7-dimethyl-*α*-tetralone (**3**) (Kongyen et al. 2015), (3*R*,4*R*)-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-napthalenone (**4**) (Borgschulte et al. 1992), (-)-(4*R*)-regiolone (**5**) (Talapatra et al. 1988), 1,8-dimethoxynaphthalene (**6**) (Allport and Bu'Lock 1960), (2*S*)-5-hydroxy-2-methyl-chroman-4-one (**7**) (Nadeau and Sorensen 2011) and (2*R**,4*R**)-3,4-dihydro-5-methoxy-2-methyl-1(2*H*)-benzopyran-4-ol (**8**) (Zheng et al. 2016) (Figure 1). Herein, we report the isolation, structure elucidation and pharmacological activities of all compounds.

2. Results and discussion

Compound **1** was obtained as a brown oil. Its molecular formula was determined by HRESIMS (*m/z* 343.1751 $[M + H]^+$, calcd 343.1751) to be $C_{17}H_{26}O_7$, including five degrees of unsaturation. In the ^1H NMR spectrum of **1**, two aromatic protons at δ_H 6.89 (1H, d, J = 8.8 Hz, H-6) and 6.68 (1H, d, J = 8.8 Hz, H-5) indicated the presence of 1,2,3,4-tetrasubstituted benzene ring. In addition, a hydroxymethyl proton signal at δ_H 4.69 (2H, s, H-12), four signals for eight methylene protons at δ_H 2.72 (2H, dd, J = 10.0, 8.0 Hz, H-7), 1.38 (2H, overlapped, H-9), 1.38

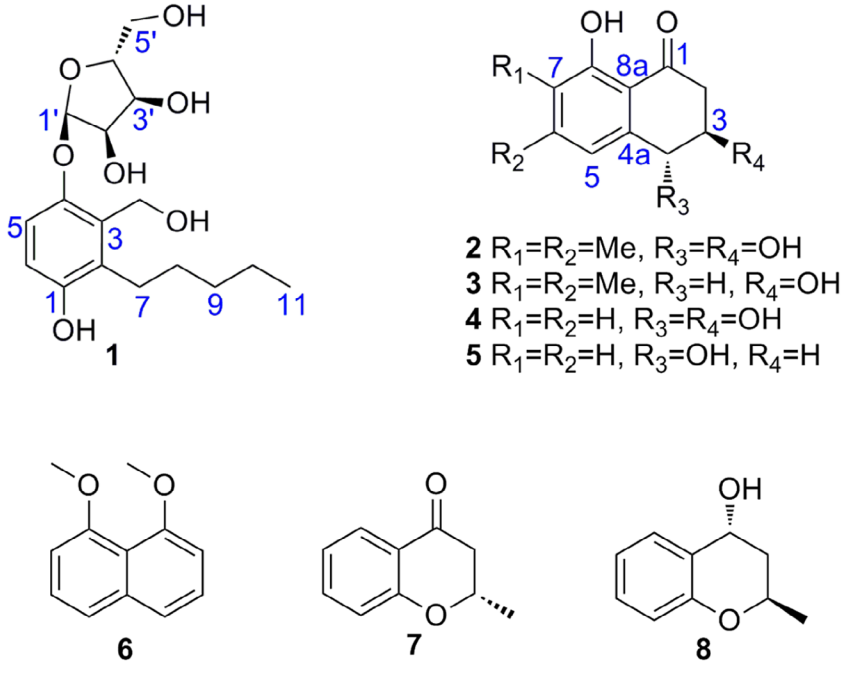

Figure 1. The structures of compounds **1**–**8**.

(2H, overlapped, H-10), 1.52 (2H, m, H-8) and four oxygenated methine proton signals at δ_H 5.55 (1H, d, J = 8.8 Hz, H-1′), 4.19 (1H, overlapped, H-4′), 4.16 (1H, overlapped, H-2′), 4.09 (1H, dd, J = 6.4, 2.4 Hz, H-3′), and two non-equivalent oxygenated methylene protons at δ_H 3.69 (1H, dd, J = 12.0, 3.6 Hz, H-5′a) and 3.65 (1H, dd, J = 12.0, 4.0 Hz, H-5′b) and one methyl group signal at δ_H 0.94 (3H, t, J = 7.2 Hz, H-11), were observed. The ^{13}C NMR and DEPT spectra (Table S1) exhibited 17 carbon resonances distributed into 1,2,3,4-tetrasubstituted benzene ring, four oxygenated methines, six methylenes including two oxygenated methylenes and one methyl group. The proton spin system [δ_H 5.55 (H-1′), 4.19 (H-4′), 4.16 (H-2′), 4.09 (H-3′), 3.69 (H-5′a) and 3.65 (H-5′b)], together with chemical shift values of the related carbons [δ_C 103.2 (C-1′), 87.9 (C-4′), 73.6 (C-2′), 71.5 (C-3′) and 63.3 (C-5′)] were typical of a ribofuranose moiety (Sharma et al. 2012).

The protons that were directly attached to carbons were assigned with the aid of HMQC spectrum. The ^{1}H–^{1}H COSY data showed proton connectivities of H-5 and H-6; of H-7, H-8, H-9, H-10 and H-11; H-1′, H-2′, H-3′, H-4′ and H-5′ (Figure S8). The HMBC correlations from H-12 to C-2, C-3 and C-4 established the attachment of the hydroxy methyl group at C-3. The long-range coupling observed H-7 to C-1, C-2 and C-3 indicated that a *n*-pentyl group was located at C-2. The HMBC correlations from H-1′ to C-4 established the attachment of the ribofuranose group at C-4 and the doublet at δ_H 5.55 (d, J = 8.8 Hz) indicated an *α* orientation of H-1′ (Sharma et al. 2012). The absolute configuration of *D*-ribose obtained from acid hydrolysis of compound **1** was established by comparison of its optical rotation ($[\alpha]_D^{25}$ −19.0, *c* 0.1, H_2O) with that of *D*-ribose previously reported (Robert et al. 1954). Hence, the compound **1** was identified as 4-*O*-*α*-*D*-ribofuranose-3-hydroxymethyl-2-pentyl- phenol.

Compound **2** was obtained as needle-like crystals. The molecular formula of **2** was determined to be $C_{12}H_{14}O_4$ on the basis of HRESIMS. The ^{1}H-NMR spectrum displayed signals for an aromatic group at δ_H 6.95 (1H, s), two oxymethine groups at δ_H 4.55 (1H, d, J = 7.2 Hz) and 4.05 (1H, ddd, J = 8.0, 7.2, 4.0 Hz), one methylene group at δ_H 3.07 (1H, dd, J = 17.2, 4.0 Hz), 2.69 (1H, dd, J = 17.2, 8.0 Hz) and two methyl singals at δ_H 2.33 (s) and 2.14 (s). The ^{13}C-NMR (Table S2) and DEPT spectra exhibited an aromatic ring (δ_C 161.3, 148.0, 142.2, 124.9, 121.3, 114.3), two methine carbons (δ_C 73.2 and 71.9), one methylene carbon (δ_C 44.3), two methyl groups (δ_C 20.9 and 10.8) and a ketone carbon (δ_C 203.8). The ^{1}H NMR and ^{13}C NMR spectra of compound **2** were similar to those of *cis*-(3*R**, 4*S**) 3,4,8-trihydroxy-6,7-dimethyl-3,4dihydronaphthalen-1(2*H*)-one (Isaka et al. 2009) expect for the coupling constant data. A small coupling constant of $^3J_{H\text{-}3,\,H\text{-}4}$ = 3.4 Hz observed in *cis*-(3*R**, 4*S**) 3,4,8-trihydroxy-6,7-dimethyl-3,4dihydronaphthalen-1(2*H*)-one, a large coupling constant of $^3J_{H\text{-}3,\,H\text{-}4}$ = 7.2 Hz was observed in **2** which showed H-3 and H-4 has *trans* diaxial relationship. Furthermore, comparing the CD data and optical rotation of **2** with those of **4** (Peng et al. 2013), they showed similar features in the 200–500 nm range (Figure S16), therefor **2** was determined as 4*R*. In conclusion, compound **2** was elucidated as (-)-*trans*-(3*R*,4*R*)-3,4,8-trihydroxy-6,7-dimethyl-3,4-dihydronaphthalen- 1(2*H*)-one.

By comparing physical and spectroscopic data with literature values, six known compounds were identified as (3*S*)-3,8-dihydroxy-6,7-dimethyl-*α*-tetralone (**3**), (-)-*trans*-(3*R*,4*R*)-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-napthalenone (**4**), (-)-(4*R*)- regiolone (**5**), 1,8-dimethoxy naphthalene (**6**), (2*S*)-5-hydroxy-2-methyl-chroman-4- one (**7**) and (2*R**,4*R**)-3,4-dihydro-5- methoxy-2-methyl-1(2*H*)-benzo pyran-4-ol (**8**) in Figure 1.

Compounds **1–3** were evaluated for cytotoxic activity against the human cervical carcinoma Hela cell line. However, in the assay all IC_{50} values obtained were higher than 10 μM

and were defined as inactive. Compounds **1**–**8** were evaluated for their antibacterial activities against six pathogenic bacteria, including *S. aureus*, *Bacillis cereus*, *Escherichia coli*, *Vibrio alginolyticus*, *Vibrio parahemolyticus*, *Methicillin-resistant Staphylococcus aureus*. The results illustrated that only **3** exhibited a broad spectrum of antibacterial activities against six pathogenic bacteria at a concentration of 20 μM.

3. Experimental

3.1. General

Melting points were determined on an X-6 micromelting point apparatus and were uncorrected. Optical rotation was measured on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Thermo Nicolet 6700 (using KBr discs) spectrophotometer (Thermo Scientific, Madison, WI, USA). 1D and 2D NMR spectra were measured on a Bruker AV-400 (Bruker Corporation, Switzerland) instrument with TMS as the internal standard. HRESIMS spectra were made on the Bruker Daltonics Apex-Ultra 7.0 T (Bruker Corporation, Billerica, MA, USA). Semi-preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C_{18} column (9.4 × 250 mm, 5 μm). Sephadex LH-20 (Pharmacia Co. Ltd, Sandwich, UK) and silica gel (200–300 mesh, 300–400 mesh Qingdao Marine Chemical Factory, Qingdao, China) were used for column chromatography (CC). All solvents were purchased from Xilong Chemical Reagent Factory (Guangzhou, China).

3.2. Fungal materials

The fungus *Cladosporium* sp. JJM22 was isolated from the mangrove plant *C. tagal*, which was collected from Dongzhaigang of Hainan Province in China in July, 2016. The strain was deposited in the Key Lab of Tropical Medicinal Plant Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, P.R. China. It was cultured in 200 mL of potato dextrose broth at 30 °C on a rotary shaker (120 rpm) for 7 days to prepare the seed culture. Large-scale fermentation was carried out in 200 Erlenmeyer flasks (1000 mL), each containing rice (65 g), sea salt (1.35 g) and purified water (45 mL), which were soaked overnight before autoclaving at 121 °C for 20 min. Those Erlenmeyer flasks were added 3-mL seed broth, respectively, after cooling to room temperature, furthermore, which were maintained at room temperature for 25 days in stationary phase.

3.3. Identification of fungus

The strain was identified as *Cladosporium* sp. according to morphologic traits and molecular identification, whose 497 base pair ITS sequence had 99% sequence identity to that of *Cladosporium* sp. The sequence data have been submitted to GenBank with the accession number MF593626.

3.4. Extraction and isolation

The fungal culture was extracted with EtOAc (3 × 20 L, 24 h each), which was filtered through cheesecloth. The extract was concentrated in vacuo to yield an oily residue (20.8 g), which

was subjected to silica gel CC (petroleum ether/EtOAc *v/v*, gradient 100:0–0:100) to generate six fractions (Fr. E1–Fr. E6). Fr. E2 was crystallised from petroleum ether/EtOAc to afford **6** (15.0 mg). Fr. E4 was concentrated and rechromatographed on a silica gel column eluted with petroleum ether/EtOAc (50:1 to 1:1) to afford seven fractions (E4-1–E4-7). Fr. E4-2 was evaporated and subjected to Sephadex LH20 column chromatography ($CHCl_3$/MeOH, *v/v*, 1:1) to afford seven fractions (E4-2-1–E4-2-7). Fr. E4-2-3 was purified by reversed-phase preparative HPLC developed with 40% acetonitrile–water at a flow rate of 1.8 mL min^{-1} to yield compounds **2** (7.0 mg), **3** (25.0 mg), **4** (11.0 mg) and **5** (13.0 mg). Fr. E5 was subjected to reversed-phase HPLC with 35% acetonitrile–water at a flow rate of 2.0 mL min^{-1} to furnish compounds **7** (15.0 mg) and **8** (17.0 mg). Compund **1** (15.0 mg) was obtained from fraction E6 using reversed-phase HPLC with 30% acetonitrile-water at a flow rate of 1.8 mL min^{-1}.

3.5. Physical properties of compounds 1 and 2

Compound **1**: brown oiliness; UV (MeOH) λ_{max} (log ε) 292 (0.262), 209 (1.071) nm; IR (KBr) ν_{max} 3373, 2928, 1649, 1460, 1043, 479 cm^{-1}; positive HRESIMS *m/z* 343.1751 $[M+H]^+$ (calcd for $C_{17}H_{27}O_7$, 343.1751). $[\alpha]_D^{25}$–9.8° (*c* 0.5 MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ: 6.89 (1H, d, *J* = 8.8 Hz, H-6), 6.68 (1H, d, *J* = 8.8 Hz, H-5), 5.55 (1H, d, *J* = 8.8 Hz, H-1′), 4.69 (2H, s, H-12), 4.19 (1H, overlapped, H-4′), 4.16 (1H, overlapped, H-2′), 4.09 (1H, dd, *J* = 6.4, 2.4 Hz, H-3′), 3.69 (1H, dd, *J* = 12.0, 3.6 Hz, H-5′a), 3.65 (1H, dd, *J* = 12.0, 4.0 Hz, H-5′b), 2.72 (2H, dd, *J* = 10.0, 8.0 Hz, H-7), 1.52 (2H, m, H-8), 1.38 (4H, overlapped, H-9,10), 0.94 (3H, t, *J* = 7.2 Hz, CH_3-11); ^{13}C NMR (100 MHz, CD_3OD) δ: 151.4 (C-1), 150.5 (C-4), 131.1 (C-3), 130.6 (C-2), 115.5 (CH-6), 115.1 (CH-5), 103.2 (CH-1′), 87.9 (CH-4′), 73.6 (CH-2′), 71.5 (CH-3′), 63.3 (CH_2-5′), 57.3 (CH_2-12), 33.2 (CH_2-9), 31.5 (CH_2-8), 27.0 (CH_2-7), 23.6 (CH_2-10), 14.4 (CH_3-11).

Compound **2**: needle-like crystals; m.p. 174.5-175.5 °C; UV (MeOH) λ_{max} (log ε) 257 (0.98), 212 (5.02) nm; IR (KBr) ν_{max} 3433, 2925, 2851, 1745, 1631, 1161, 520 cm^{-1}; HRESIMS *m/z* 223.0971 $[M + H]^+$ (Calcd 223.0964, $C_{12}H_{15}O_4$); $[\alpha]_D^{25}$–35° (*c* 0.1 MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ: 6.95 (1H, s, H-5), 4.55 (1H, d, *J* = 7.2 Hz, H-4), 4.05 (1H, ddd, *J* = 8.0, 7.2, 4.0 Hz, H-3), 3.07 (1H, dd, *J* = 17.2, 4.0 Hz, H-2b), 2.69 (1H, dd, *J* = 17.2, 8.0 Hz, H-2a), 2.33 (3H, s, 9-CH_3), 2.14 (3H, s, CH_3-10); ^{13}C NMR (100 MHz, CD_3OD) δ: 203.8 (C-1), 161.3 (C-8), 148.0 (C-4a), 142.2 (C-6), 124.9 (C-8a), 121.3 (C-5), 114.3 (C-7), 73.2 (CH-4), 71.9 (CH-3), 44.3 (CH_2-2), 20.9 (CH_3-9), 10.8 (CH_3-10); CD (*c* 0.5 MeOH) $\Delta\varepsilon$ −10.28 (217.6 nm), +3.18 (266.9 nm).

3.6. Acid hydrolysis of 1 and determination of the configuration of the ribofuranose

A solution of **1** (10 mg) in 6 mol/L HCl (1 mL) was reacted 3 h at 100 °C. The reaction mixture was extracted with EtOAc repeatedly to remove the aglycone fraction. The H_2O layer was then concentrated to furnish the sugar residue (2.0 mg). The rotation recorded for the ribose isolated in this study was $[\alpha]_D^{25}$–19.0 (*c* 0.1, H_2O), which closely matched that for the *D*-ribose (lit.–20) (Lin et al. 1995).

3.7. Biological assays

Cytotoxic activity was evaluated by the MTT method as described previously. Human cervical carcinoma Hela cell line was used. Epirubicin was used as a positive control (Scudiero et al.

1988). Antibacterial activity was determined against six terrestrial pathogenic bacteria, including *S. aureus* (ATCC 6538), *E. coli* (ATCC 25,922), *B. cereus* (ATCC 6633), *V. alginolyticus* (ATCC 33,787), *V. parahemolyticus* (ATCC 17,802), MRSA (CMCC(B) 63,303) (Pierce et al. 2008). Ciprofloxacin was used as the positive control.

4. Conclusions

In this study, one new ribofuranose phenol derivative 4-*O*-*α*-*D*-ribofuranose-3- hydroxymethyl-2-pentylphenol (**1**) and one new naphthalene derivative (-)-*trans*-(3*R*,4*R*)-3,4,8-trihydroxy-6,7-dimethyl-3,4dihydronaphthalen-1(2*H*)-one (**2**), together with six known compounds (**3–8**), were obtained from the EtOAc extract of *Cladosporium* sp. JJM22, an endophytic fungus isolated from the stem bark of the mangrove plant *C. tagal* collected in South China Sea. The absolute configuration of *D*-ribose obtained from acid hydrolysis of compound **1** was established by comparison of its optical rotation with that of *D*-ribose previously reported. The absolute configuration of **2** was confirmed by the comparison of CD spectrum analysis with the known compound **4**.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S15, Tables S1 and S2.

Disclosure statement

No potential conflict of interest was reported by the authors.

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