Polyketides From the Endophytic Fungus Cladosporium sp. Isolated From the Mangrove Plant Excoecaria agallocha

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Five new polyketides (2–6) and ten known compounds (1 and 7–15) were obtained from the fermentation products of the endophytic fungus Cladosporium sp. OUCMDZ-302 with the mangrove plant, Excoecaria agallocha (Euphorbiaceae). The new structures of 2–6 were established on the basis of ECD, specific rotation and spectroscopic data as well as the chemical calculation. Compound 7 showed cytotoxicity against H1975 cell line with an IC50 value of 10.0 μM. Compounds 4 and 8–10 showed radical scavenging activity against DPPH with the IC50 values of 2.65, 0.24, 5.66, and 6.67 μM, respectively. In addition, the absolute configuration of compound 1 was solidly determined by X-ray and sugar analysis of the acidic hydrolysates for the first time as well as those of compounds 8–10 in this paper.

Keywords: Cladosporium sp., mangrove fungus, Excoecaria agallocha, polyketides, anti-oxidation

INTRODUCTION

Mangrove plants and endophytic fungi are two principal sources of new and bioactive natural products (Zhang et al., 2006; Wu et al., 2008). Excoecaria agallocha (Euphorbiaceae), also known as blind-your-eye, is mainly used to treat sores and stings. More than 72 cytotoxic diterpenoids have been identified from E. agallocha, structurally belonging to labdane (Konishi et al., 1999, 2003; Anjaneyulu and Rao, 2000; Annam et al., 2015), isopimarane/ent-isopimarane (Anjaneyulu et al., 2003; Wang and Guo, 2004; Kang et al., 2005; Wang et al., 2005; Gowri Ponnapalli et al., 2013), atisane/ent-atisane (Konishi et al., 2000; Wang et al., 2009), ent-kaurane (Anjaneyulu et al., 2002; Li et al., 2010), and beyerane-type (Anjaneyulu et al., 2002).

In our ongoing investigations of new and bioactive compounds from endophytes associated with mangrove plants (Lin et al., 2008; Lu et al., 2009, 2010; Wang et al., 2012; Kong et al., 2014; Zhu et al., 2018), an endogenous fungal strain OUCMDZ-302 identified as Cladosporium sp., was isolated from the surface-sterilized stems of E. agallocha. The secondary metabolites of the genus Cladosporium were mainly reported as polyketides derivatives, such as macrolides (Jadulco et al., 2001; Zhang et al., 2001; Shigemori et al., 2004), α-pyrones (Jadulco et al., 2002), α-pyridone (Ye et al., 2005), and binaphthyl derivatives (Sakagami et al., 1995).
Herein we report five new polyketides (2–6) (Figure 1) isolated from the EtOAc extract of Cladosporium sp. OUCMDZ-302, along with the ten known structures (Figure S36 and Table S1), (2R)-7-O-α-D-ribofuranosyl-5-hydroxy-2-methylchroman-4-one (1) (Hu et al., 2017), 7-O-α-D-ribosyl-5-hydroxy-2-propylchromone (7) (Zhao et al., 2015), 3-(2,3-dihydroxy phenoxy)butanoic acid (8) (Dai et al., 2009), (2S,4S)-4-methoxy-2-methylchroman-5-ol (9) (Wu et al., 2010), (2S,4S)-2-methylchroman-4,5-diol (10) (Teles et al., 2005), (±)-5-dihydroxy-2-methyl chroman-4-one (11) (Rao et al., 1994), (±)-5-dihydroxy-2-methylchroman-4-one (12) (Dai et al., 2006), 1-(2,6-dihydroxyphenyl) ethanone (13) (Dhami and Stothers, 1965), 1-(2,6-dihydroxyphenyl)-1-butanone (14) (Huang et al., 2005), and 2-butyryl-3,5-dihydroxycyclohex-2-enone (15) (Igarashi et al., 1993). Compound 7 showed inhibitory activity against H1975 cell line with an IC50 value of 10.0 µM. Compounds 4 and 8–10 exhibited radical scavenging activity against DPPH with IC50 values of 2.65, 0.24, 5.66, and 6.67 µM, respectively. In addition, the absolute configurations of compounds 8–10 were resolved and that 1 was solidified in this paper.

**Materials and Methods**

**General Experimental Procedures**

The NMR, ECD, [α]D, UV and IR spectra were recorded on JEOL JNM-ECP 600, JASCO J-810, JASCO P-1020 digital, Beckman DU® 640 and Nicolet NEXUS 470 spectrophotometers, respectively. ESI-MS, EI-MS and GC-MS were measured on Q-TOF ULTIMA GLOBAL GAA076 LC, VG Autospec-3000 and Agilent 6890/5973 spectrometers, respectively. Semipreparative HPLC and chiral separation was performed on a YMC-pack ODS-A column [10 × 250 mm, 5 µm, 4 mL/min] and a CHIRALPAK IA column [20 × 250 mm, 5 µm, 10 mL/min]. TLC was performed on plates precoated with silica gel GF254 (10–40 µm). The column chromatography (CC) was performed over silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively. The seawater for the cultural medium of Cladosporium sp. OUCMDZ-302 was collected from Yellow Sea near Qingdao.

**Fungal Material**

The strain Cladosporium sp. OUCMDZ-302 was isolated from the surface sterilized stems of the mangrove plant E. agallocha grown in Wenchang, Hainan, China. Briefly, the stems were washed with tap water and sterile distilled water in sequence. The stems with clean surface were further sterilized in a sequence of 75% ethanol for 2 min, 0.1% of HgCl2 for 3 min, and sterile distilled water. The outer bark was removed, and the inner bark was cut into small pieces that were then placed on a potato dextrose agar (PDA) plate and cultured at 28°C for 3 days. A single colony was transferred to PDA media and was identified according to its morphological characteristics (Figure S35) by Prof. Kui Hong, Wuhan University. A voucher specimen is deposited in our laboratory at −80°C. The working strain was prepared on PDA slants and stored at 4°C.

**Fermentation and Extraction**

The producing fungal strain Cladosporium sp. OUCMDZ-302 was inoculated into a 500 mL cylindrical flask containing 100 mL of seawater consisting of 2% maltose, 2% mannitol, 1% glucose, 1% monosodium glutamate, 0.3% yeast extract, 0.1% corn flour, 0.05% KH2PO4, 0.03% MgSO4·7H2O (pH 6.5) and cultured at 28°C for 48 h on a rotary shaker at 120 rpm. The seed culture was transferred into three hundred and fifty 500 mL conical flasks (200 mL/flask) containing the same medium, and performed at 28°C for 7 days on rotary shakers at 160 rpm. The whole fermentation broth (70 L) was filtered through cheese cloth to separate the mycelia from filtrate. The filtrate was concentrated to about one-quarter of the original volume under reduced pressure and then extracted three times with equal volumes of ethyl acetate (EtOAc) and concentrated to dryness. The mycelia were extracted three times with acetone and concentrated to an aqueous solution. The aqueous solution was subsequently extracted three times with equal volumes of EtOAc and concentrated. Both EtOAc extractions were combined to give 45 g of the extract.

**Isolation**

The extract (45 g) was separated into eight fractions (Fr.1–Fr.8) on a silica gel column (8.5 × 15 cm, 200–300 mesh) using a step gradient elution with CHCl3-petroleum ether (V/V 1:100–100:1, 4 L) and then MeOH–CHCl3 (V/V 0:100–100:0, 16 L). Fr.1 (5.4 g) was separated on a silica gel column (4.5×10 cm, 200–300 mesh) eluted with CHCl3-petroleum ether (V/V, 1: 1, 3L) to give 12 (1g). Fr.3 (0.3 g) was further purified by semipreparative HPLC (60% MeOH/H2O) to yield 10 (7 mg, tR 4.97 min). Fr.4 (4.3 g) was separated into two subfractions by column chromatography over silica gel (RP-18) eluting with gradient H2O-MeOH (50–100%). Fr.4-1 (1.4 g) was separated by Sephadex LH-20 (3 × 75 cm, MeOH, 300 mL) to obtain three fractions (130 mL, Fr.4-1-1; 90 mL, Fr.4-1-2; 80 mL, Fr.4-1-3). Fr.4-1-2 (140 mg) was purified by semipreparative HPLC (30% MeOH/H2O) to yield 5 (1 mg, tR 8.24 min), 13 (30 mg, tR 20.20 min), and 15 (5 mg, tR 18.15 min). Fr.4-1-3 (190 mg) was purified by semipreparative HPLC (30% MeOH/H2O, 0.15% CF3CO2H) to yield 4 (15 mg, tR 16.76 min) and 8 (30 mg, tR 14.41 min). Fr.4-2 (360 mg) was purified by semipreparative HPLC (50% MeOH/H2O) to give 6 (10 mg, tR 12.28 min), and 14 (24 mg, tR 18.12 min). Fr.5 (1.1 g) was separated into two subfractions by a silica gel column (2.6 × 10 cm, 200–300 mesh) eluted with MeOH–CHCl3 (V/V 1:40, 1L). Fr.5-1 (40 mg) was purified by semipreparative HPLC (25% MeOH/H2O) to yield 3 (3 mg, tR 6.76 min), and Fr.5-2 (80 mg) was purified by semipreparative HPLC (50% MeOH/H2O, 0.15% CF3CO2H) to give compounds 11 (5 mg, tR 6.87 min). Fr.6 (2.8 g) was separated into two subfractions by a silica gel column (4.5 × 10 cm, 200–300 mesh) eluted with CHCl3-petroleum ether MeOH–CHCl3 (V/V 1:25, 2L). Fr.6-1 (110 mg) was purified by semipreparative HPLC (60% MeOH/H2O) to give 9 (10 mg, tR 10.28 min). Fr.6-2 (340 mg) was purified by semipreparative HPLC (50% MeOH/H2O) to give 7 (18 mg, tR 13.55 min) and the mixture of 1 and 2 (70 mg, tR 5.56 min).
MeOH–MeCN–EtOH 40:40:20) to yield compound 1 (35.4 mg, \( t_R \) 8.22 min) and 2 (23.7 mg, \( t_R \) 4.69 min).

**ECD, [\( \alpha \)]D and Coupling Constant Calculation**

Calculations for ECD and [\( \alpha \)]D were performed in HyperChem 7.5 and Gaussian 03 (Frisch et al., 2004; Chen et al., 2016; Jin et al., 2018). Karplus formula was used to compute the coupling constant (\( J \)) from the proton-proton torsion angle (Haasnoot et al., 1980).

**Cytotoxic Assays**

Cytotoxicities of compounds 1–14 against HL-60 and K562 cell lines were assayed by the MTT method (Mosmann, 1983), while those for BEL-7402, A549, HeLa, and H1975 cell lines were tested by SRB (Skehan et al., 1990) methods. Adriamycin was used as the positive control with the IC50 values of 0.02, 0.21, 0.48, 1.32, 0.32, and 0.38, respectively.

**Anti-oxidant Activities**

The anti-oxidant activities of compounds 1–14 were evaluated by DPPH assay in vitro (Wang et al., 2007). Vitamin C was used as the positive control with an IC50 value of 3.29 \( \mu \)M.

**Antimicrobial Assays**

The antimicrobial activities of compounds 1–14 against *E. coli*, *E. aerogenosa*, *B. subtilis*, and *C. albicans* were evaluated by an agar dilution method (Zaika, 1988). Ciprofloxacin lactate and ketoconazole was used as the positive controls for bacteria and fungi with MIC values of 4.0, 0.5, 32.0, 16.0, 4.1 \( \mu \)g/mL, respectively.

**RESULTS AND DISCUSSION**

**Identification of Compounds**

Compounds 1 and 2 were first isolated as an isomeric mixture whose molecular formula was determined to be \( C_{13}H_{16}O_8 \) by HRESIMS at \( m/z \ 327.1068 \) [M+H]\(^+\) (calcd 327.1080), indicating seven degrees of unsaturation. An interpretation of the 1D (Table 1, Figures S1–S3) and 2D NMR (Figure 2 and Figures S4–S6) spectra established a pentose moiety and a benzopyrane moiety similar to those of 5,7-dihydroxy-2-methylchroman-4-one (11) (Rao et al., 1994). The upfield shift of C-7 (\(-1.5 \) ppm) and the key HMBC correlations between the anomeric proton (\( \delta_H \) 1.5/5.66/5.68) and C-7 (\( \delta_H \) 165.1/165.0) indicated that 1 and 2 were 7-O-pentosides of 11. Acetic hydrolysis of the mixture of 1 and 2 with 2 \( M \) HCl yielded \((\pm )-11\) and D-ribose that was identified by GC-MS analysis of the reaction products with L-cysteine methyl ester and Me3SiCl (Figures S33, S34) (Deyrup et al., 2007). These data indicated that 1 and 2 are a pair of epimers at C-2. Separation of 2-epimeric mixture of 1 and 2 was achieved on a chiral column using MeOH-MeCN-EtOH as eluent. And then, NMR data of optically-pure 1 (Figures S7, S8) and 2 (Figures S9, S10) were obtained. X-ray single crystal diffraction of 1 revealed the \( \alpha \)-glycosidic bond and 2R-configuration (Figure 3). The ECD Cotton effects of compounds 1 and 2 were opposite in sign (Figure 4), confirming the opposite configuration of C-2. Thus, the structures of 1 and 2 were unambiguously elucidated as (2R)- and (2S)-7-\( \alpha \)-D-ribofuranosyl-5-hydroxy-2-methylchroman-4-one, respectively. This is the first time that to solidify the absolute configuration of compound 1, although it was reported last year (Hu et al., 2017).

The molecular formula of compound 3 was determined to be \( C_{19}H_{12}O_4 \) based on the HRESIMS peak at \( m/z \ 195.0659 \) [M–\( H \)]\(^+\) (calcd 195.0657), indicating five degrees of unsaturation. The NMR data (Table 1, Figures S11–S14) was similar to those of 10 (Teles et al., 2005), except for the upfield methylene signal at \( \delta_H \) 1.50 & 1.88/38.0 that was replaced by the one of an oxygenated methine at \( \delta_H \) 3.45/69.8. This was further supported by the \( ^1H–^1H \) COSY from H-9 (\( \delta_H \) 3.131) to H-4 (\( \delta_H \) 4.48) through H-2 (\( \delta_H \) 4.14) and H-3 (\( \delta_H \) 3.45) (Figure 2 and Figure S15), and the key HMBC correlations of H-9 to C-3 (\( \delta_H \) 69.8) and H-3 to C-4a (\( \delta_H \) 111.2) (Figure 2 and Figure S16). In order to confirm the relative configuration, we calculated the coupling constant of H2-H3 and H3-H4 for the four possible relative configurations 3A–3D (Figure 5). The computational \( ^3J \) value of 3A was most near to the measured result (Table 3).
The absolute configuration was established by calculation of the specific rotation. The measured [α]D value of 3 (−53.6) is consistent with the calculated one for (2S,3S,4R)-3 (−100) and opposite to the calculated one for (2R,3R,4S)-3 (+102). Thus, the structure of 3 was identified as (2S,3S,4R)-2-methylchroman-3,4,5-triol.

Compound 4 showed the molecular formulae of C_{11}H_{14}O_{5} based on HREIMS peaks at m/z 225.0767 [M−H]− (calcd 225.0763), indicating five degrees of unsaturation. The 1D (Figures S17−S19) and HMBC (Figure S20) NMR spectra of 4 displayed three sp² methines and four sp³ quaternary carbon signals, one sp³ oxygenated methine signals, one sp³ methylene signals and two methyl group (including one methoxy). The 1D NMR data (Table 1) of 4 were almost identical to those of 8 [Figure S1; (Dai et al., 2009)] except for an additional methoxy (δ_{H/C} 3.78/52.5) and the upfield shift for carbonyl carbon (−3.5 ppm), indicating that 4 is the methyl ester of 8. This was confirmed by analysis of ¹H−¹H COSY correlation (Figure S21) and the key HMBC between the methoxy protons at δ_{H} 3.78 and the carbonyl carbon at δ_{C−1} 174.0 (Figure 2 and Figure S22). The specific rotations of both 4 ([α]D +14.4) and 8 ([α]D +8.2) were opposite to the synthetic analog, R-3-(3-methoxyphenyloxy)butanoic acid ([α]D =−31.2) (Kawasaki et al., 2008), indicating both 4 and 8 as S-configuration. The S-configuration of 4 was also backed by the coincidence of experimental and calculated ECD curves (Figure 6). Thus, the structure of compounds 4 and 8 were established as methyl (3S)-3-(2,3-dihydroxyphenyloxy) butanoate and (3S)-3-(2,3-dihydroxyphenyloxy)butanoic acid, respectively.

The molecular formula of compound 5 was determined as C_{7}H_{12}O_{2} based on the HREIMS peak at m/z 128.0845 [M]⁺ (calcd 128.0837), corresponding to two degrees of unsaturation. The IR spectrum showed hydroxy groups at 3442 cm⁻¹ and double bonds at 3080 and 1646 cm⁻¹. The 1D NMR spectra
(Table 2, Figures S23–S25) of 5 showed two double bonds one of which is terminal, two oxygenated methines and one methyl group (Table 1). These groups were connected to the full structure of CH$_2$ =CH−CH=CH−CH (OH)−CH (OH)−CH$_3$ on the basis of $^1$H−$^1$H COSY correlations from the methyl ($\delta_H$ 1.01) to the methylene ($\delta_H$ 5.03/5.17) through the two oxygenated methines (Figure 2 and Figure S26). The large value of $^3$J$_{H-4,H-5}$ (15.4 Hz) corresponded to E-$\Delta^4$ double bond. The large $^3$J$_{H-2,H-3}$ value (6.0 Hz) and the downfield methyl carbon signal ($\delta_C$ 19.1) indicated an anti-conformation (Jarvis et al., 1996; Zhang and O’doherty, 2005; Nilewski et al., 2009), corresponding to threo-configuration of 2,3-diol (Zheng et al., 2010). In order to confirm the relative configuration of compound 5, $^3$J$_{H-2,H-3}$ of threo-5 and erythro-5 were computed. The results showed that the predicted $^3$J$_{H-2,H-3}$ values of threo-5 (5.5 Hz) matched with the measured one (6.0 Hz) while the calculated one of erythro-5 (3.8 Hz) was inconsistent, indicating threo-configuration. The direction of the specific rotation of 5 ($[\alpha]_D$ $-$6.8) were similar to the structurally related t-butyl (6S,7S)-6,7-dihydroxyocta-2,4-dienoate ($[\alpha]_D$ $-$23) (Zhang and O’doherty, 2005), and opposite to t-butyl (6R,7R)-6,7-dihydroxyocta-2,4-dienoate ($[\alpha]_D$ +22.9) (Zhang and O’doherty, 2005). The structure of 5 was thus deduced as (2S,3S,4E)-hepta-4,6-diene-2,3-diol.

The molecular formula of compound 6 was determined to be C$_{11}$H$_{18}$O$_2$ based on the HREIMS peak at m/z 182.1305 [M]$^+$ (calcd. 182.1307), indicating three degrees of unsaturation. The EIMS of 6 illustrated in Figure 7 indicates the existence of
-CH₂OH, -C₆H₅O, and -C₅H₆O moieties. The ¹H (Figure S27) and ¹³C (Figure S28) NMR spectra and DEPT (Figure S29) and HMOC (Figure S30) experiments of 6 revealed 11 signals including three double bonds one of which is terminal, one oxygenated methine, four methylenes one of which is oxygenated. The ¹H-¹H COSY (Figure S31) correlations from H-1 (δ 3.65) to H-11 (δ 4.14/5.02) in sequence established the structure, CH₂=CH–CH=CH₂-CH(OH)-CH₂-CH=CH₂-CH₂-CH₂OH, which was supported by HMBC correlations (Figure S32). The large values of 3J₃H–H₄ (15.4 Hz) and 3J₅H–H₉ (14.8 Hz) suggested that both Δ³ and Δ⁸ double bonds were E-configurations. The direction of specific rotation of 6 ([α]D +2.0) is similar to that of (S)-dodeca-3,5-diene-1,7-diol ([α]D +56) (Zhang and Kyler, 1989), suggesting S-configuration at C-6. Thus, the structure of 6 was deduced as (3E,8E,6S)-undeca-3,8,10-triene-1,6-diol.

The relative configurations of compounds 9 and 10 were determined as (-)-trans-4-methoxy-2-methylchroman-5-ol (Wu et al., 2010) and (-)-trans-2-methyl chroman-4-5-diol (Teles et al., 2005), respectively. The absolute configuration of compound 9 was determined by quantum chemical ECD calculation. The measured ECD of 9 was coincident with the calculated ECD of (2S,4R)-9 (Figure 8). Thus, compound 9 was established to be (2S,4S)-4-methoxy-2-methyl chroman-5-ol. The similar sign of the specific rotations of 9 and 10 ([α]D²⁻ = −2.0 vs. [α]D²⁺ = −6.0, MeOH) suggests the same absolute configuration. Therefore, compound 10 was determined to be (2S,4S)-2-methylchroman-4,5-diol. The absolute configurations of compounds 9 and 10 were determined for the first time in this study.

(2R)-7-O-α-D-Ribofuranosyl-5-hydroxy-2-methylchroman-4-one (1): White amorphous powder; [α]D²⁺ = +198.9 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 204 (3.62), 278 (3.55), 320 (2.77) nm; ECD (MeOH) λmax (Δε) 211 (+14.21), 284 (−3.51), 327 (+3.42); IR (KBr) νmax 3416, 1646, 1573, 1354, 1295, 1195, 1155, 1076, 1029 cm⁻¹; ¹H and ¹³C NMR (Table 1); HRESIMS m/z 327.1068 [M+H]+ (calcd for C₁₃H₁₉O₈ 327.1080).

(2S)-7-O-α-D-Ribofuranosyl-5-hydroxy-2-methylchroman-4-one (2): White amorphous powder; [α]D²⁺ = +118.6 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 204 (3.62), 278 (3.55), 320 (2.77) nm; ECD (MeOH) λmax (Δε) 211 (−10.04), 284 (+9.40), 320 (−2.13); IR (KBr) νmax 3416, 1646, 1573, 1354, 1295, 1195, 1155, 1076, 1029 cm⁻¹; ¹H and ¹³C NMR (Table 1); HRESIMS m/z 327.1068 [M+H]+ (calcd for C₁₃H₁₉O₈ 327.1080).

(2S,3S,4R)-2-Methylchroman-3,4,5-triol (3): Colorless oil; [α]D²⁺ = −3.6 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 200 (3.25), 270 (2.26) nm; IR (KBr) νmax 3429, 2356, 1627, 1401, 1090 cm⁻¹; ¹H and ¹³C NMR (Table 1); HRESIMS m/z 195.0659 [M–H]⁻ (calcd for C₁₀H₁₁O₄ 195.0657).

Methyl (3S)-3-(2,3-dihydroxyphenylxy)butanoate (4): Colorless oil; [α]D²⁺ = +14.4 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 200 (3.32), 270 (2.19) nm; IR (KBr) νmax 3409, 2356, 1706, 1606, 1481, 1202, 1063, 1010 cm⁻¹; ¹H and ¹³C NMR (Table 1); HRESIMS m/z 225.0767 [M–H]⁻ (calcd for C₁₃H₂₁O₅ 225.0763).

(2S,3S,4E)-Hepta-4,6-diene-2,3-diol (5): Colorless oil; [α]D²⁺ = −6.8 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 200 (3.09), 217 (3.38) nm; IR (KBr) νmax 3442, 3080, 1646, 1540, 1023, 446 cm⁻¹; ¹H and ¹³C NMR (Table 2); EIMS m/z (%): 129 (45), 256 (8), 111 (26), 97 (51), 83 (69), 82 (38); HREIMS m/z 128.0845 [M]+ (calcd for C₉H₁₅O₂ 128.0837).

(3E,8E,6S)-Undeca-3,8,10-triene-1,6-diol (6): Colorless oil; [α]D²⁺ = +2.0 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 200 (2.86), 218 (3.16) nm; IR (KBr) νmax 3390, 2927, 1715, 1421, 1047, 973 cm⁻¹; ¹H and ¹³C NMR (Table 2); EIMS m/z (%): 181 (8), 164 (9), 151 (14), 129 (17), 115 (16), 97 (31), 85 (28), 71...
FIGURE 9 | Possible biosynthetic pathway of the compounds 1–15.

Biogenetic Origin

These compounds were postulated to be biosynthesized by the polyketide pathway from acetyl coenzyme A (Figure 9). The acetyl-CoA units underwent condensation, cyclization, dehydration and hydrogenation to produce compounds 11 and 12. Compound 11 formed compounds 1 and 2 by glycosidation. (S)-12 underwent oxidation and reduction to yield compound 3. The reduction of (S)-12 produced compound 10 that was transformed to compound 9 followed by methylation. (S)-12 was subjected to Baeyer-Villiger oxidation followed by methanolysis and hydrolysis to yield compounds 4 and 8, respectively. Compounds 5 and 6 were formed from different lengths of acetyl-CoA units by condensation, reduction, dehydration, and decarboxylation. The condensation of acetyl-CoA units followed by cyclization and reduction formed compound 15 that was transformed to compound 14 after enolization and dehydration.

Biological Activity

Compounds 1–14 were tested for cytotoxic effects on the HL-60, BEL-7402, K562, A549, HeLa, and H1975 cell lines, DPPH scavenging activity, and antimicrobial activities against E. coli, E. aerogenes, P. aeruginosa, B. subtilis, and C. albicans. As the results, compound 6 was cytotoxic to...
H1975 cell line with an IC_{50} values of 10.0 \mu M, while compounds 4 and 8–10 showed DPPH radical scavenging activity with the IC_{50} values of 2.65, 0.24, 5.66, and 6.67 \mu M, respectively. None of the compounds exhibit antimicrobial activities.

### CONCLUSIONS

Five new polyketides were isolated and identified from the fermentation of the mangrove fungus Cladosporium sp. OUCMDZ-302 with Excoecaria agallocha. The new compound 4 showed DPPH radical scavenging activity with an IC_{50} value of 2.65 \mu M.

### AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fchem.2018.00344/full#supplementary-material
Polyketides From the Mangrove Fungus Cladosporium sp.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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