Bioorganic & Medicinal Chemistry Letters 20 (2010) 3326–3328

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The metabolites of mangrove endophytic fungus Zh6-B1 from the South China Sea

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ARTICLE INFO

Article history:
Received 11 January 2010
Revised 11 March 2010
Accepted 10 April 2010
Available online 14 April 2010

Keywords:
Fungi
Metabolites
Isolation

ABSTRACT

Two new metabolites, 3*R*,5*R*-Sonnerlactone (**1**) and 3*R*,5*S*-Sonnerlactone (**2**), were isolated from the mangrove endophytic fungus Zh6-B1 obtained from the South China Sea. Their structures were elucidated by MS and NMR. The absolute configuration of compound **1** was determined by single-crystal X-ray analysis using Cu Kα radiation. The absolute configuration of compound **2** was determined by NOESY analysis and comparing circular dichroism spectroscopy with compound **1**. The antiproliferative activity of compound **1** and **2** against the multi-drug resistant human oral floor carcinoma cells (KV) was evaluated.

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Study showed that a number of novel cytotoxic metabolites have been discovered from marine-derived fungi.[1] Mangrove on South China Sea coast was an important resource of marine-derived fungi and many novel bioactive metabolites have been isolated from it.[2] The endophytic fungus strain Zh6-B1 was isolated from the bark of *Sonneratia apetala* from Zhu Hai, Guangdong, China. The species of this fungus was unidentified. In this Letter, the metabolites of this fungus were investigated. Two new metabolites, 3*R*,5*R*-Sonnerlactone (**1**) and 3*R*,5*S*-Sonnerlactone (**2**) and two known compounds, 3,4-dihydro-4,8-dihydroxy-7-(2-hydroxyethyl)-6-methoxy-1(2*H*)-naphthalen-1-one (**3**) and 10-Norparvulenone (**4**) were isolated (Fig. 1).

The extract of fungus Zh6-B1[3] was subjected to silica gel column chromatography (200–300 mesh), which was eluted with a gradient of petroleum ether (60–90 °C) and ethyl acetate, and then with a gradient of ethyl acetate and methanol. The fraction containing compound **1** and **2** was further purified by HPLC (YMC ODS-A column, 10 × 250 mm, 5 μm) using methanol/water (60:40, v/v) as eluent, to obtain colorless crystals **1** (150 mg) and white powder **2** (120 mg). The fraction containing compound **3** and **4** was further purified by HPLC using methanol/water (40:60, v/v) as eluent, to obtain colorless crystals **3** (8 mg) and white powder **4** (30 mg).

According to HREIMS analysis, compound **1**[4] had a molecular formula $C_{14}H_{18}O_5$. The ^{1}H NMR spectrum (Table 1) indicated the presence of one methyl group (1.44 ppm, d, J = 6.4 Hz) attached to methine and one tetra-substituted benzene ring (6.22 ppm and 6.26 ppm). Three D_2O-exchangeable protons at 3.64 ppm, 9.07 ppm and 11.03 ppm indicated the presence of two phenolic OH and one OH attached to aliphatic carbon. The ^{13}C NMR spectrum of **1** revealed the presence of one ester carbonyl carbon (171.94 ppm). The DEPT spectra indicated the presence of four methylene groups, four methine groups and five quaternary carbons. The correlations of CH_3/3-H/4-H/5-H/6-H/7-H/8-H in ^{1}H–^{1}H COSY spectrum indicated the presence of an aliphatic chain of

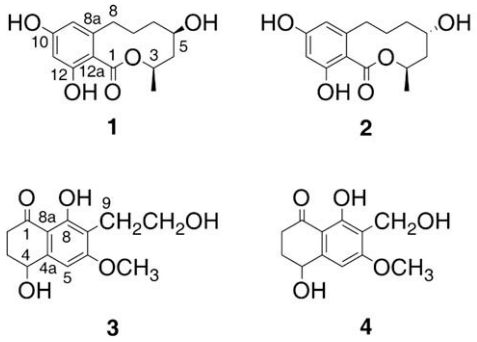

Figure 1. Compounds **1–4** isolated from mangrove endophytic fungus Zh6-B1.

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doi:10.1016/j.bmcl.2010.04.036

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

Position	Compound **1**		Compound **2**	
	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1	171.94, qC		172.68, qC	
3	73.25, CH	4.95, m	71.43, CH	5.41, m
4	45.91, CH_2	2.02, dd (6.0, 2.8)	43.98, CH_2	1.88, ddd (14.8, 3.6, 1.2)
				2.15, ddd (14.8, 6.8, 4.8)
5	72.01, CH	3.85, m	67.91, CH	4.23, m
6	37.10, CH_2	1.53, m	38.26, CH_2	1.48, m
		1.79, m		1.76, m
7	27.08, CH_2	1.31, m	29.56, CH_2	1.48, m
		2.09, m		1.80, m
8	37.58, CH_2	2.57, ddd (11.6, 8.0, 5.0)	37.29, CH_2	2.41 dt (12.8, 8.0)
		3.24, dt (12.8, 7.2)		3.39, ddd (12.8, 4.4, 4.0)
8a	149.51, qC		150.91, qC	
9	111.86, CH	6.26, d (2.4)	113.08, CH	6.29, d (2.4)
10	163.37, qC		164.56, qC	
11	101.75, CH	6.22, d (2.4)	102.77, CH	6.24, d (2.4)
12	165.41, qC		167.61, qC	
12a	106.24, qC		106.34, qC	
3-CH_3	21.47, CH_3	1.44, d (6.4)	21.22, CH_3	1.45, d (6.8)
5-OH		3.64, br s		3.66, br s
10-OH		9.07, br s		9.08, br s
12-OH		11.03, br s		11.56, br s

CH_3/C-3/C-4/C-5/C-6/C-7/C-8. In the HMBC spectrum of **1** (Fig. 2), the correlation of 9-H, 11-H/C-1 indicated that the carbonyl carbon of ester group was connected with phenyl group. The multiplet signal of 3-H at 4.95 ppm indicated that the C-3 was connected with the O atom of ester group. The correlations of 8-H/C-8a, C-9 indicated that the C-8 was connected with phenyl group. The correlations of 9-H/C-10, C-11 and 11-H/C-10, C-12, C-12a indicated the substituted form of phenyl ring. In the NOESY spectrum, the correlation between 3-H and 5-H indicated that 3-Me and 5-OH of compound **1** was at the same side. According to single-crystal X-ray analysis using Cu Kα radiation,[5] the absolute configuration of compound **1** (Fig. 3) was confirmed and could be assigned as 3*R*,5*R*-Sonnerlactone. The CD spectrum of compound **1** (Fig. 4) showed positive Cotton effect at 266 nm for $\pi \rightarrow \pi^*$ excitation of the ester chromophore. It is contrary to those of the similar compounds xestodecalactones A, B, and C,[6] who had the opposite configuration at 3-Me.

According to HREIMS analysis, compound **2**[7] had a same molecular formula as compound **1**, $C_{14}H_{18}O_5$. Furthermore, compound **2** had similar ^{1}H NMR, ^{13}C NMR, and DEPT spectra data as compound **1** (Table 1), which indicated that compound **2** had a same skeleton structure as compound **1**. In the NOESY spectrum of compound **2**, no correlation between 3-H and 5-H was observed, which indicated that they were at the different side. As shown in Figure 4, the CD spectrum of compound **2** also showed positive Cotton effect at 266 nm which indicated that the configuration of 3-Me of compound **2** was the same as compound **1**. Therefore, compound **2** was assigned as 3*R*,5*S*-Sonnerlactone.

Two known compounds, **3** and **4**,[8,9] were identified by NMR, MS analysis and comparison of spectroscopic data with literatures.[10,11]

The antiproliferative activity of compounds **1** and **2** was evaluated against multi-drug resistant human oral floor carcinoma cells (KV/MDR) by MTT method.[12,13] The harvested KV/MDR cells were exposed to various concentrations of the compounds for 48 h. The results indicated that compounds **1** and **2** could inhibit the KV/MDR growth by 42.4% and 41.6%, respectively, at 100 μM.

In summary, we investigated the metabolites of marine-derived fungus Zh6-B1, and found two new lactones together with two

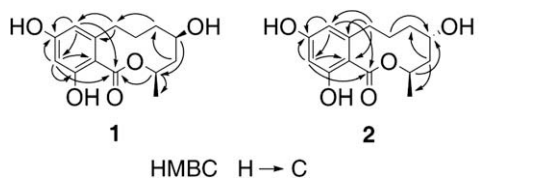

Figure 2. Key HMBC correlations of compounds **1** and **2**.

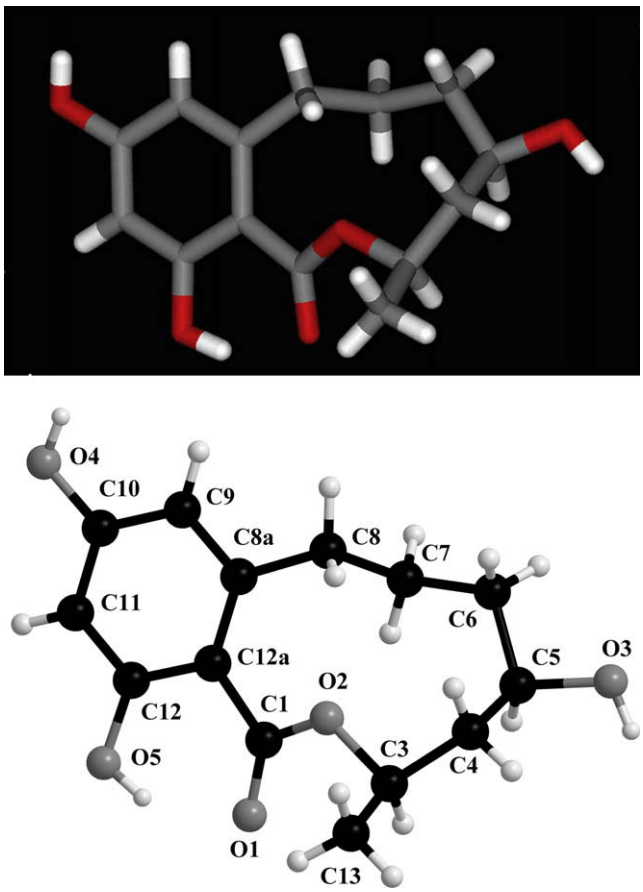

Figure 3. Perspective ORTEP drawing of compound **1**.

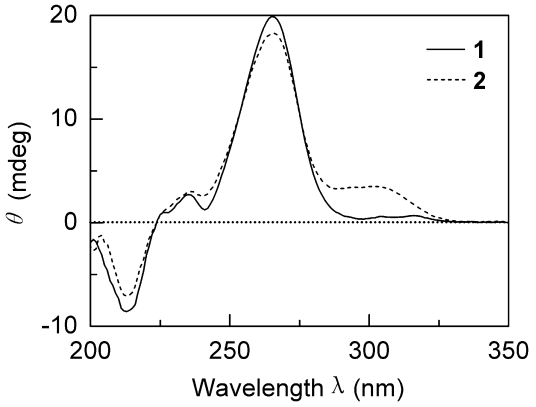

Figure 4. CD spectra of compounds **1** and **2**.

known compounds. The two lactones showed cytotoxic activity against KV/MDR cell line. They might possess beneficial therapeutic potential against drug-resistant tumors.

Acknowledgments

We thank Professor Yue-Ming Li, College of Pharmacy, Nankai University for his helpful instruction in crystallography.

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3. *The extract preparation of fungus Zh6-B1*: the fungus was cultured stationary in a 1 L Erlenmeyer flask containing 500 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 3 g/L) at 20 °C for three months. The cultures (200 L) were filtered through cheesecloth. The filtrate was concentrated to 10 L below 40 °C and extracted with equal volume of ethyl acetate for three times. The ethyl acetate layer was concentrated to little volume.
4. *Spectral data of compound* **1**: $C_{14}H_{18}O_5$, colorless hexagonal crystals; mp 146–147 °C; $[\alpha]_D^{20}$ +9.0 (*c* 1.0, EtOH); UV (EtOH) λ_{max} (log ε) 214 (4.42), 266 (4.13), 301 (3.76) nm; CD (MeOH) λ(mdeg): 213 (−8.58), 226 (0.98), 235 (2.71), 266 (19.90) nm; IR (KBr) ν_{max}: 3531, 3506, 3424, 3379, 3180, 2949, 1657, 1603, 1465, 1454, 1394, 1322, 1271, 1212, 1166, 1136, 1076 cm^{-1}. HREIMS *m/z* $[M]^+$ 266.1149, calcd 266.1149.
5. 3*R*,5*R*-Sonnerlactone (**1**) was crystallized from MeOH/H_2O as colorless hexagonal crystals. Molecular formula = $C_{14}H_{20}O_6$, molecular mass = 284.30 amu. Crystal system = trigonal. Space group = *P*3(2)21. Unit cell dimensions *a* = 8.72829(16) Å, *b* = 8.72829(16) Å, *c* = 63.1374(11), α = 90, β = 90, γ = 120. cell volume = 4165.58(13) $Å^3$. Density = 1.360 mg/m^3. *F*(0 0 0) = 1824. λ(Cu Kα) = 1.5418 Å at 150 K. *Z* = 15. A total of 49565 refections were recorded of which 5225 reflections were judged on the basis of $I > 2\sigma(I)$. The structure was solved by direct methods and all calculations were performed using the SHELXL PC program. The final *R* and *Rw* factors were 0.0500 and 0.1118, respectively. Crystallographic data for compound **1** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 758115. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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7. *Spectral data of compound* **2**: $C_{14}H_{18}O_5$, white powder; mp 159–160 °C; $[\alpha]_D^{20}$ +64.0 (*c* 1.0, EtOH); UV(EtOH) λ_{max} (log ε) 215 (4.43), 266 (4.16), 302 (3.79) nm; CD (MeOH) λ(mdeg): 213 (−7.07), 236 (3.00), 266 (18.28), 303 (3.50) nm; IR (KBr) ν_{max}: 3524, 3450, 3202, 2962, 2935, 1651, 1623, 1586, 1467, 1384, 1357, 1342, 1314, 1269, 1171, 1130, 1092, 1076, 1054, 988 cm^{-1}. HREIMS *m/z* $[M]^+$ 266.1149, calcd 266.1149.
8. *Data of compound* **3**: 3,4-Dihydro-4,8-dihydroxy-7-(2-hydroxyethyl)-6-methoxy-1(2*H*)-naphthalen-1-one, $C_{13}H_{16}O_5$, white powder; mp 167–168 °C; HREIMS *m/z* $[M]^+$ 238.0837, calcd 238.0836; ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 1.93 (1H, m, 3-H), 2.17 (1H, m, 3-H), 2.68 (2H, m, 2-H), 2.74 (2H, m, 9-H), 3.41 (2H, m, 10-H), 3.89 (3H, s, 6-OCH_3), 4.55 (1H, t, *J* = 5.2 Hz, 10-OH), 4.71 (1H, m, 4-H), 5.57 (1H, d, *J* = 5.6 Hz, 4-OH), 6.78 (1H, s, 5-H), 12.97 (1H, s, 8-OH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 25.97 (C-9), 31.53 (C-3), 34.95 (C-2), 55.85 (6-OCH_3), 59.30 (C-10), 66.81 (C-4), 100.67 (C-5), 109.43 (C-8a), 111.46 (C-7), 148.69 (C-4a), 161.31 (C-8), 163.54 (C-6), 203.15 (C-1).
9. Spectral data of compound **4**: 10-Norparvulenone, $C_{12}H_{14}O_5$, colorless block crystals; mp 150–151 °C; $[\alpha]_D^{25}$ -27.0 (*c* 1.0, EtOH); UV (EtOH) λ_{max} (log ε) 224 (4.39), 284 (4.14), 325 (3.74) nm; HREIMS *m/z* $[M]^+$ 238.0837, calcd 238.0836; ^{1}H NMR (CD_3COCD_3, 400 MHz) δ_H 2.07 (1H, m, 3-H), 2.30 (1H, m, 3-H), 2.66 (1H, ddd, *J* = 17.6, 10.4, 4.8 Hz, 2-H), 2.81 (1H, ddd, *J* = 17.6, 6.4, 4.8 Hz, 2-H), 3.38 (1H, t, *J* = 6.4 Hz, 4-OH), 3.94 (3H, s, 6-OCH_3), 4.63 (1H, t, *J* = 6.4 Hz, 9-OH), 4.65 (2H, d, *J* = 6.4 Hz, 9-H), 4.86 (1H, m, 4-H), 6.85 (1H, s, 5-H), 13.12 (1H, s, 8-OH); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ_C 32.66 (C-3), 35.77 (C-2), 52.75 (C-9), 56.33 (6-OCH_3), 68.05 (C-4), 101.53 (C-5), 110.76 (C-8a), 116.18 (C-7), 150.92 (C-4a), 163.17 (C-8), 165.05 (C-6), 203.95 (C-1).
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