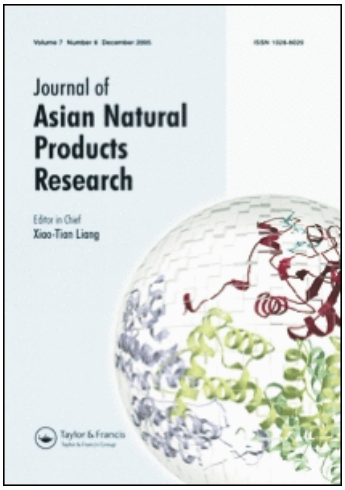

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Two new compounds from fermentation liquid of the marine fungus *Trichoderma atroviride* G20-12

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The chemical constituent research on the ethyl acetate extracts of fermentation liquid of the marine fungus *Trichoderma atroviride* G20-12 led to the isolation of two new compounds, 2-hydroxybutan-3-yl 5′-(2″-hydroxy-*N*-(2‴-oxobutan-3‴-yl)propanamido)butanoate (**1**) and 3-hydroxy-5-(4-hydroxybenzyl)dihydrofuran-2(3*H*)-one (**2**). The structures of the new compounds were determined by spectroscopic and chemical analysis.

Keywords: chemical constituent; marine fungus; *Trichoderma atroviride*

1. Introduction

Marine micro-organisms have been proven to be rich sources of bioactive secondary metabolites, and numerous compounds with potent biological and unique chemical structures have been isolated [1]. As an important component of marine micro-organisms, the diversity of marine fungal metabolites is given growing recognition [2]. In this investigation, we report the metabolites from the mangrove marine fungus G20-12 separated from the sediment on the root of *Ceriops tagal*, which is arbor collected at the South Sea intertidal zone and identified as *Trichoderma atroviride*. Chromatographic separation led to the isolation of two new compounds, 2-hydroxybutan-3-yl 5′-(2″-hydroxy-*N*-(2‴-oxobutan-3‴-yl)propanamido)butanoate (**1**) and 3-hydroxy-5-(4-hydroxybenzyl)dihydrofuran-2(3*H*)-one (**2**) (Figure 1). The structures of these compounds were determined by spectroscopic and chemical analysis.

2. Results and discussion

Compound **1** was obtained as a colorless oil. The molecular formula was determined as $C_{15}H_{27}NO_6$ by HR-FAB-MS at *m/z* 318.1920 $[M+H]^+$. The IR spectrum of **1** showed absorption bands at 3425, 1734, 1715, and 1651 cm^{-1}, indicating the presence of hydroxyl, ester carbonyl, ketone, and aminocarbonyl groups, which were supported by the observation of the UV absorption maximum (λ_{max} 206 nm). The ^1H NMR (600 MHz, DMSO-d_6) spectrum exhibited five methyl signals at δ 1.00 (3H, d, $J = 6.6$ Hz, H-1), 1.09 (3H, d, $J = 6.6$ Hz, H-4), 1.19 (3H, d, $J = 6.6$ Hz, H-4‴), 1.23 (3H, d, $J = 6.6$ Hz, H-1″), and 2.05 (3H, s, H-1‴), two oxygenated methine signals at δ 3.59 (1H, m, H-2) and 4.08 (1H, m, H-2″), which showed correlations with carbons at δ 67.3 (C-2)

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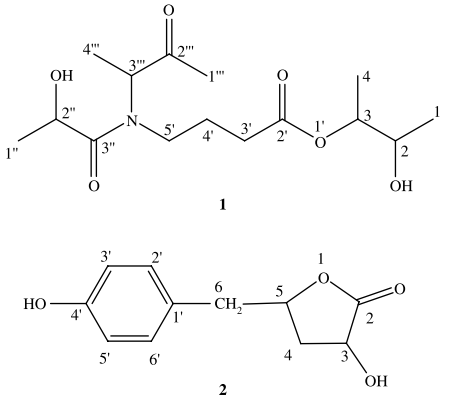

Figure 1. The structures of compounds **1** and **2**.

and 66.1 (C-2″) in the HMQC spectrum, and the downfield chemical shift at δ 2.05 (3H, s, H-1‴) suggested the presence of a neighboring keto group. The protons at δ 2.25 (2H, d, $J = 7.8$ Hz, H-3′), 1.94 (2H, m, H-4′), 3.21 (1H, m, H-5′a), and 3.31 (1H, m, H-5′b) suggested the presence of a fragment consisting of three methylenes. The ^{13}C NMR spectrum (150 MHz, DMSO-d_6) exhibited three carbonyl carbon signals at δ 206.5 (C-2‴), 174.3 (C-2′), and 174.2 (C-3″). In the HMQC spectrum, no correlation between protons at δ 4.73 (1H, d, $J = 6.0$ Hz) and 5.30 (1H, d, $J = 6.0$ Hz) and any carbon was observed, which indicated that the protons are active hydrogen. In the HMBC spectrum, the protons at δ 1.00 (3H, d, $J = 6.6$ Hz) and 1.09 (3H, d, $J = 6.6$ Hz) showed correlations with C-2 and C-3, respectively (Figure 2). The proton at δ 4.73 (1H, d, $J = 6.0$ Hz) showed correlations with C-1 and C-3, which suggested the existence of fragment **1a** (Figure 3). The proton at δ 2.25 (2H, d, $J = 7.8$ Hz, H-3′) showed correlations with C-4′, C-5′, and C-2′, while the protons at δ 3.21 (1H, m, H-5′a) and 3.31 (1H, m, H-5′b) showed correlations with C-4′, C-3′, and C-3″, which suggested the existence of fragment **1b**. Fragment **1b** was located at C-3 by the protons at δ 4.66 (1H, m, H-3) correlating with C-2′. The proton at δ 1.23 (3H, d, $J = 6.6$ Hz, H-1″) showed HMBC correlations with C-2″ and C-3″, and H-2″ showed correlations with C-1″ and C-3″, which could lead to fragment **1c**. The protons at δ 1.19 (3H, d, $J = 6.6$ Hz, H-4‴) and 2.05 (3H, s, H-1‴) showed correlations with C-3‴ and C-2‴, which

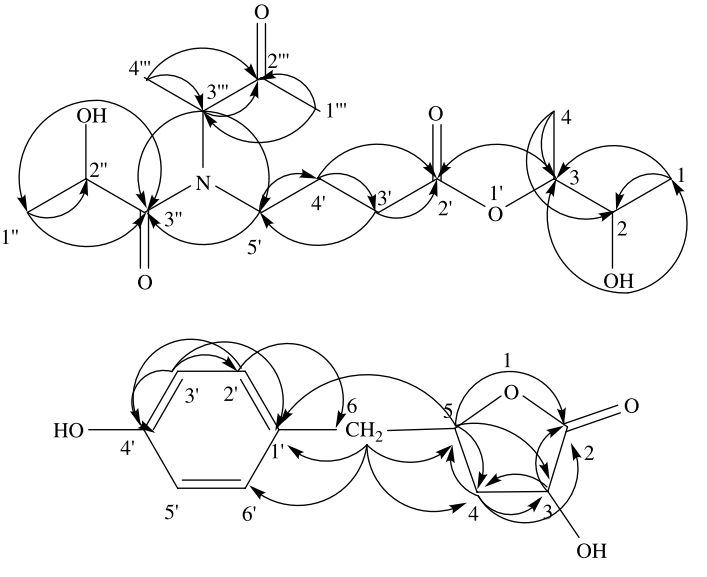

Figure 2. The key HMBC correlations of compounds **1** and **2**.

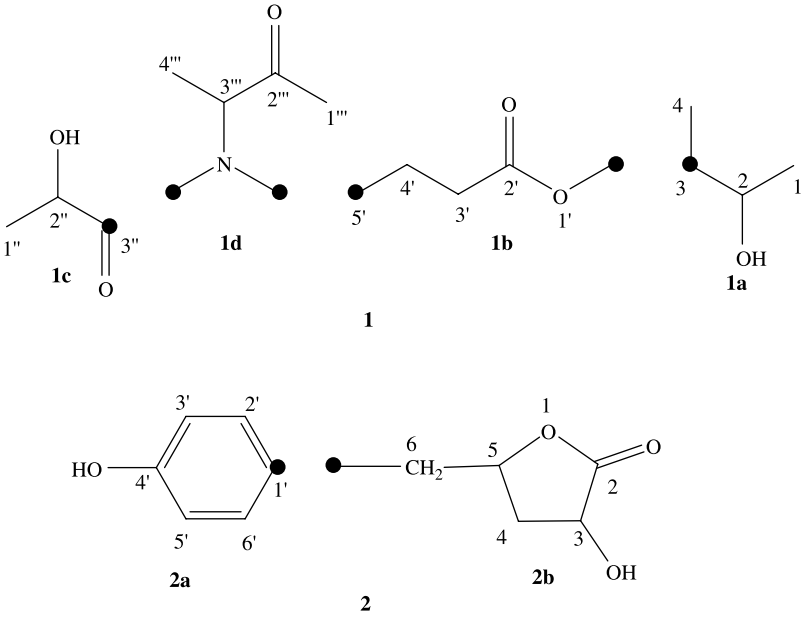

Figure 3. The fragments of compounds **1** and **2**.

suggested the existence of fragment **1d**. The proton at δ 4.47 (1H, q, $J = 6.6$ Hz, H-3‴) showed correlations with C-5′ and C-3″; the protons at δ 3.21 (1H, m, H-5′a) and 3.31 (1H, m, H-5′b) showed correlations with C-3″, and the compound has an N atom. Therefore, the structure of compound **1** was confirmed as 2-hydroxybutan-3-yl 5′-(2″-hydroxy-*N*-(2‴-oxobutan-3‴-yl)propanamido)butanoate.

Compound **2** was obtained as a colorless oil and gave a positive reaction with the $FeCl_3$ reagent. The molecular formula was determined as $C_{11}H_{12}O_4$ by HR-FAB-MS at *m/z* 209.0815 $[M+H]^+$. The IR spectrum showed absorption bands for the hydroxyl group (3401 cm^{-1}) and benzene skeleton (1609, 1523, 1461 cm^{-1}), which was supported by the observation of the UV spectral data (λ_{max} 277 nm). The ^1H NMR (300 MHz, DMSO-d_6) spectrum exhibited a phenolic hydroxyl at δ 9.30 (4′-OH). Four aromatic protons at δ 6.69 (2H, d, $J = 8.4$ Hz, H-3′, 5′) and 7.00 (2H, d, $J = 8.4$ Hz, H-2′, 6′) were observed, indicating a *para*-substituted benzene. The ^{13}C NMR (75 MHz, DMSO-d_6) spectrum established the presence of one ester carbonyl carbon at δ 177.1 (C-2). In the HMBC experiment, the proton at δ 4.02 (H, t, $J = 7.8$ Hz, H-3) showed correlations with C-2, C-4, and C-5 (Figure 2), while the proton at δ 2.04 and 2.20 showed correlations with C-2, C-3, C-5, and C-6, indicating the presence of fragment **2b** (Figure 3). The proton at δ 7.00 (2H, d, $J = 8.4$ Hz, H-2′, 6′) showed correlations with C-4′, C-3′, 5′, and C-6. Additionally, the proton at δ 2.79 (1H, d, H-6) showed correlations with C-4, C-5, C-1′, C-2′, and C-6′. These data revealed that fragment **2b** was located at C-1′. Therefore, the structure of compound **2** was confirmed as 3-hydroxy-5-(4-hydroxybenzyl)dihydrofuran-2(3*H*)-one.

3. Experimental

3.1 General experimental procedures

UV spectra were recorded on a Shimadzu UV-1601. IR spectra were recorded on a

Bruker IFS-55 infrared spectrophotometer. The NMR data were recorded on a Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) and a Bruker DRX-300 instrument (300 MHz for ^{1}H and 75 MHz for ^{13}C) in DMSO-d_6 with TMS as the internal standard. The HR-FAB-MS data were obtained using the Micross Mass Autospec-Ultima ETOF mass spectrophotometer. Chromatography was performed on silica gel (200–300 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, NJ, USA), and reversed-phase HPLC (Shimadzu LC-8A vp, Kyoto, Japan).

3.2 Fungal strain

The fungal strain was isolated from the sediment on the root of the mangrove *C. tagal*, which was arbor collected at the South Sea intertidal zone, China, in 2005, and identified as *T. atroviride* by DNA extractions and PCR amplifications by Prof. Li Tian. Moreover, the strain was recorded at GenBank with the code number FJ481096. A voucher specimen (No. HTTM-Z05005) has been deposited in the Key Laboratory of Marine Biology of the First Institute of Oceanography, State Oceanography Administration SOA, Qingdao, China.

3.3 Culture conditions

The strain was cultured on seed medium at 24°C on a rotary shaker for 5 days. The culture medium contained 200 ml potato decoction, 2.0 g peptone, 1.0 g yeast powder, 20.0 g dextrose, 17.0 g NaCl, 1.0 g $MgCl_2{\cdot}6H_2O$, 0.1 g KCl, 0.01 g $FePO_4$, and 1000 ml distilled water at 24°C on a rotary (150 rpm) shaker for 15 days. On the 15th day, the fermentation broth, including the cells, was harvested (Figures 4 and 5).

3.4 Extraction and isolation of metabolites

The supernatant of the fermentation broth (65 liters) was concentrated to 5 liters *in vacuo* and extracted three times with an equal volume of ethyl acetate and *n*-butanol, successively. The ethyl acetate extract (about 26 g) was chromatographed on silica gel column using a gradient

Figure 4. The characteristics of conidiophores and conidia of G20-12 strain.

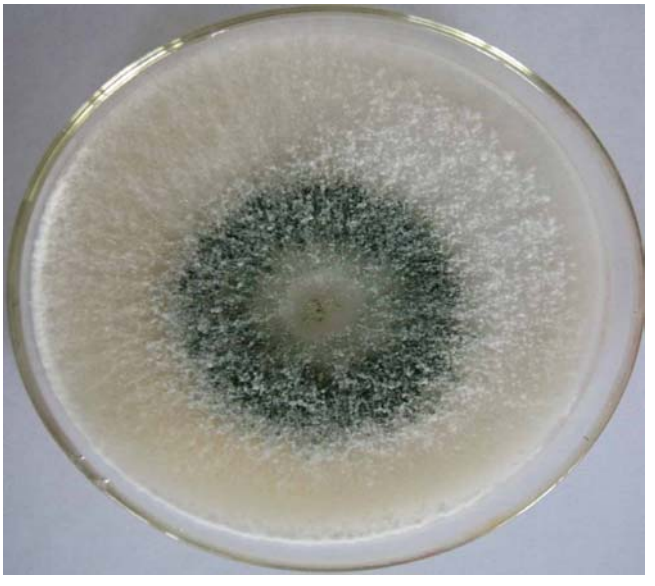

Figure 5. The colonial morphology of G20-12 strain.

elution with $CHCl_3$–MeOH (100:0–0:100) to afford 10 fractions (F1–10). Fraction 2 was subjected to column chromatography on silica gel and eluted with petroleum ether–acetone (100:1–1:1) to give 10 subfractions. Subfraction 8 was purified by preparative HPLC (MeOH–H_2O 30:70 v/v; flow rate 4 ml/min; UV detection 210 nm) to afford compound **1** (6 mg, 26.3 min). Subfraction 7 was subjected to Sephadex LH-20 column chromatography, eluting with MeOH and further separated by preparative HPLC (MeOH–H_2O 40:60 v/v; flow rate 4 ml/min; UV detection 210 nm) to afford compound **2** (11 mg, 34.7 min).

Table 1. ^1H and ^{13}C NMR spectral data of compound **1** in DMSO-d_6 (150 MHz for ^{13}C, 600 MHz for ^1H).

No.	δ_C	δ_H
1	18.5	1.00 (3H, d, $J = 6.6$ Hz)
2	67.3	3.59 (1H, m)
3	73.7	4.66 (1H, m)
4	15.3	1.09 (3H, d, $J = 6.6$ Hz)
2′	174.3	–
3′	30.4	2.25 (2H, d, $J = 7.8$ Hz)
4′	17.9	1.94 (2H, m)
5′	43.7	3.21 (1H, m), 3.31 (1H, m)
1″	20.5	1.23 (3H, d, $J = 6.6$ Hz)
2″	66.1	4.08 (1H, m)
3″	174.2	–
1‴	26.6	2.05 (3H, s)
2‴	206.5	–
3‴	55.8	4.47 (1H, q, $J = 6.6$ Hz)
4‴	12.8	1.19 (3H, d, $J = 6.6$ Hz)
2-OH	–	4.73 (1H, d, $J = 6.0$ Hz)
2″-OH	–	5.30 (1H, d, $J = 6.0$ Hz)

Table 2. ^1H and ^{13}C NMR data of compound **2** in DMSO-d_6 (75 MHz for ^{13}C, 300 MHz for ^1H).

No.	δ_C	δ_H
1	–	–
2	177.1	–
3	66.1	4.02 (1H, t, $J = 7.8$ Hz)
4	35.0	2.04 (1H, m), 2.20 (1H, m)
5	77.8	4.72 (1H, m)
6	39.5	2.79 (1H, d)
1′	126.6	–
2′, 6′	130.5	7.00 (2H, d, $J = 8.4$ Hz)
3′, 5′	115.3	6.69 (2H, d, $J = 8.4$ Hz)
4′	156.1	–
3-OH	–	5.89 (1H, s)
4′-OH	–	9.30 (1H, s)

3.4.1 Compound **1**

Colorless oil (6 mg). UV (MeOH) λ_{max}: 206 nm; IR (KBr) ν_{max} (cm^{-1}): 3425, 1734, 1715, 1651; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectral data: see Table 1; HR-FAB-MS *m/z*: 318.1920 $[M+H]^+$ (calcd for $C_{15}H_{28}NO_6$, 318.1917).

3.4.2 Compound **2**

Colorless oil (11 mg). UV (MeOH) λ_{max}: 277 nm; IR (KBr) ν_{max} (cm^{-1}): 3401, 1609, 1523, 1461; ^1H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectral data: see Table 2; HR-FAB-MS *m/z*: 209.0815 $[M+H]^+$ (calcd for $C_{11}H_{13}O_4$, 209.0814).

Acknowledgements

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