SHORT REPORT

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Two New Trienoic Acid Derivatives from Marine-derived Fungus *Penicillium oxalicum* BTBU20213011

Xinyu Liu [1], Yifei Dong [1], Xinwan Zhang [2], Xinjun Zhang [3], Caixia Chen [4], Fuhang Song [1*] and Xiuli Xu [2*]

[1] *Key Laboratory of Geriatric Nutrition and Health, Ministry of Education of China; School of Light Industry, Beijing Technology and Business University, Beijing, 100048, P. R.China*

[2] *School of Ocean Sciences, China University of Geosciences, Beijing, 100083, P. R. China*

[3] *Institute of Tibet Plateau Ecology, Key Laboratory of Forest Ecology in Tibet Plateau (Ministry of Education), Tibet Agriculture & Animal Husbandry University, Nyingchi, Tibet 860000, P. R. China*

[4] *School of Medicine, University of Pittsburgh. Pittsburgh, PA 15213, USA*

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Abstract: Two new trienoic acid derivatives, namely penioxa acids A (**1**) and B (**2**), have been isolated from the marine-derived fungus strain *Penicillium oxalicum* BTBU20213011. Their structures were determined by extensive analysis of spectroscopic data, including 1D and 2D NMR, and HRESIMS.

Keywords: *Penicillium oxalicum* BTBU20213011; trienoic acid derivatives; marine-derived fungus.

1. Microorganism Material

The fungal strain BTBU20213011 was isolated from a marine sediment sample collected from the Western Pacific at a depth of 3000 m and identified as *Penicillium oxalicum* by comparing its ITS sequence with data in GenBank by using nucleotide BLAST. The ITS sequence showed 99.30% similarity to that of *Penicillium oxalicum* CBS 219.30 (accession number: MH885125). A frozen specimen (NO. BTBU20213011) preserved in 20% glycerol was deposited in Beijing Technology and Business University, Beijing, China.

2. Previous Studies

Compared to terrestrial environments, marine environments, especially the deep-sea, harbor a variety of extremely environments, such as lack of light and oxygen, high hydrostatic pressure, extreme pH and low temperature. These conditions offer significant potential for the production of

* Corresponding author: E-Mail: xuxl@cugb.edu.cn (X.Xu) ; songfuhang@btbu.edu.cn (F.Song).

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novel natural compounds. Marine-derived fungi, in order to adapt to such harsh environments, produce structurally unique secondary metabolites [1]. Over 1,000 new compounds are identified from marine organism per year [2, 3]. The genus of *Penicillium*, which comprises approximately 354 species [4,5] and is widely distributed in the natural environment, serves as a prolific source of bioactive natural products [6]. Various compounds, including secalonic acids H–M, penoxahydrazones A–C, emodin-3-*O*-sulphate and citreorosein-3-*O*-sulphate, have been identified from *P. oxalicum* strains derived from marine mud or sediment samples [7-11].

3. Present Study

Colonies of the BTBU20213011 strain were inoculated into a 250 mL Erlenmeyer flask containing 50 mL potato dextrose broth (PDB) medium. The flask was then incubated in a shaker (180 rpm, 28 °C) for three days to generate the seed broth. 5mL of the seed broth were transferred into 20 seperate 1 L flasks, each containing 150 g rice and 120 mL distilled H_2O. All the flasks were incubated at 28 °C for 30 days in a static state.

The fermented materials were extracted three times using a mixture of EtOAc/MeOH (80/20) as the solvent. The combined extracts were concentrated under vacuum to yield a crude extract which was further partitioned three times with 500 mL EtOAc/H_2O (50/50) to afford an EtOAc residue (16.84 g). The EtOAc extract was then subjected to column chromatography (normal phase silica gel column, 50 × 80 mm), eluted with a mixture of hexane/CH_2Cl_2 and CH_2Cl_2/MeOH to yield 16 fractions (*Fr.1−Fr.16*). Fraction *Fr.13* was further purified by on Sephadex LH-20 column eluted with CH_2Cl_2:MeOH (2:1) to give sixteen subfractions Fr.13.1-Fr.13.16. Subfraction Fr.13.6 was separated by using an Agilent 1200 HPLC system equipped with an Eclipse XDB-C18 column (250 × 9.4 mm, 5 μm) to obtain **1** (4.3 mg) and **2** (10.6 mg). The mobile phase consisted of an isocratic elution of 20% MeCN over 10 min, followed by a gradient increase to 40% MeCN over 10 min, then to 60% MeCN over 20 min, and finally to 100 MeCN over 15 min. the flow rate was set at 3.0 mL/min.

*Penioxa acid A **(1)***: Light yellow gum; $[\alpha]_D^{25}$ = – 11.0 (*c* = 0.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.04 (3H, *d*, *J* = 6.5 Hz, H-13), 1.12 (3H, *d*, *J* = 6.0 Hz, H-10), 1.87 (3H, *d*, *J* = 1.0 Hz, H-12), 1.94 (3H, *s*, H-11), 2.54 (1H, *ddq*, *J* = 10.0, 6.5, 6.5 Hz, H-8), 3.55 (1H, *dq*, *J* = 6.5, 6.0 Hz, H-9), 5.57 (1H, *d*, *J* = 10.0 Hz, H-7), 6.48 (1H, *dd*, *J* = 15.0, 11.0 Hz, H-4), 6.60 (1H, *d*, *J* = 15.0 Hz, H-5), 7.26 (1H, *d*, *J* = 11.0 Hz, H-3); ^{13}C NMR (125 MHz, CD_3OD): δ (ppm) = 12.8 (CH_3, C-12), 12.9 (CH_3, C-11), 16.9 (CH_3, C-13), 21.4 (CH_3, C-10), 42.1 (CH, C-8), 72.6 (CH, C-9), 123.0 (CH, C-4), 127.0 (C, C-2), 135.1 (C, C-6),140.6 (CH, C-3), 141.3 (CH, C-7), 145.9 (CH, C-5), 172.0 (C, C-1).

*Penioxa acid B **(2)***: Light yellow gum; $[\alpha]_D^{25}$ = – 5.0 (*c* = 0.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) = 1.02 (3H, *d*, *J* = 7.0 Hz, H-13), 1.13 (3H, *d*, *J* = 6.5 Hz, H-10), 1.80 (3H, *d*, *J* = 1.0 Hz, H-12), 1.94 (3H, *d*, *J* = 1.5 Hz, H-11), 2.60 (1H, *m*, H-8), 3.67 (1H, *dq*, *J* = 6.5, 6.0 Hz, H-9), 5.66 (1H, *d*, *J* = 10.0 Hz, H-7), 6.48 (1H, *dd*, *J* = 15.0, 11.0 Hz, H-4), 6.62 (1H, *d*, *J* = 15.0 Hz, H-5), 7.26 (1H, *dd*, *J* = 11.0, 0.5 Hz, H-3); ^{13}C NMR (125 MHz, CD_3OD): δ (ppm) = 12.7 (CH_3, C-11), 12.8 (CH_3, C-12), 16.9 (CH_3, C-13), 20.7 (CH_3, C-10), 41.4 (CH, C-8), 72.2 (CH, C-9), 122.8 (CH, C-4), 126.9 (C, C-2), 135.7 (C, C-6),140.7 (CH, C-3), 140.9 (CH, C-7), 146.1 (CH, C-5), 172.0 (C, C-1).

Bioactivity Test: The antimicrobial activity was evaluated followed the antimicrobial susceptibility testing standards outlined by the Clinical and Laboratory Standards Institute document M07-A7 and previous reports [12-15]. The microdilution method was employed in a sterilized 96-well plate to test the activity against *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25923, and *S. aureus* ATCC 25923.

The EtOAc residue was subjected to normal phase silica gel column chromatography, Sephadex LH-20 column chromatography, and HPLC purification to result in the isolation of two new trienoic acid derivatives, penioxa acids A (**1**) and B (**2**) (Figure 1).

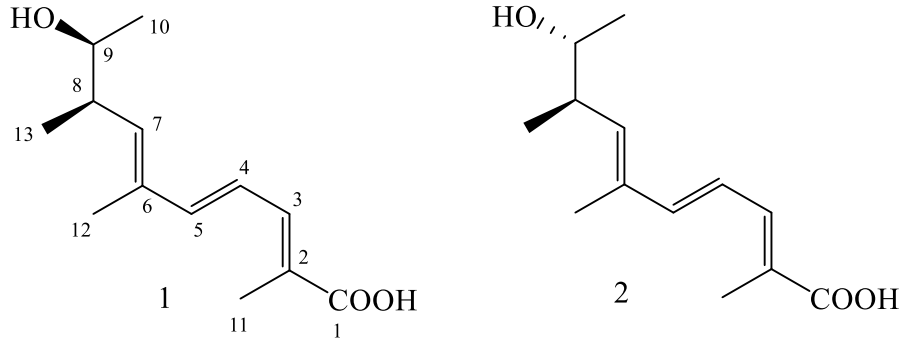

Figure 1. Structures of penioxa acids A (**1**) and B (**2**) isolated from *P. oxalicum* BTBU20213011

Compound **1** was isolated as yellow gum. The molecular formula was determined to be $C_{13}H_{20}O_3$ with four degrees of unsaturation by HR-ESI-MS (*m/z* 225.1491 $[M + H]^+$, calcd. 225.1485). The ^1H NMR of compound **1** revealed the presence of four olefinic protons (at δ_H 7.26, 1H, *d*, *J*=11.0 Hz, H-3; 6.60, 1H, *d*, *J*=15.0 Hz, H-5; 6.48, 1H, *dd*, *J*=15.0, 11.0 Hz, H-4; 5.57, 1H, *d*, *J*=10.0 Hz, H-7), two sp^3 methines (at δ_H 3.55,1H, *ddq*, *J*=10.0, 6.5, 6.0 Hz, H-9; 2.54,1H, *dq*, *J*=6.5, 6.0 Hz, H-8), three doublet methyl groups (at δ_H 1.12, *d*, *J*=6.0 Hz, H_3-10; 1.87, *d*, *J*=1.0 Hz, H_3-12; 1.04, *d*, *J*=6.5 Hz, H_3-13), as well as one singlet methyl group (at δ_H 1.94, s, H_3-11). The ^{13}C NMR and HSQC spectra of **1** confirmed the presence of thirteen signals corresponding to four methyls, two sp^3 methines (one being oxygenated), four sp^2 methines, and three sp^2 quaternary carbon atoms, including a carbonyl carbon (at δ_C 172.1, C-1). The ^1H-^1H COSY correlations (Figure 2) revealed the substructures of C-3/C-4/C-5 and C-7/C-8(C-13)/C-9/C-10, which also were further confirmed by the HMBC correlations from H_3-10 to C-8 and C-9, and from H_3-13 to C-7, C-8 and C-9. The attachment of C-1, C-3 and C-11 to C-2 was confirmed by the HMBC correlations (Figure 2) from H_3-11 to C-1, C-2 and C-3, as well as from H-3 to C-1 and C-11. Additionally, the HMBC correlations from H_3-12 to C-5, C-6 and C-7 suggested connection between C-5 and C-6. Therefore, the planar structure of **1** was determined. The coupling constant between H-3 and H-4 was 11.0 Hz, which revealed the *cis* conformation between H-3 and H-4. The geometry configurations of the double bonds between C-2 and C-3, as well as between C-4 and C-5 were identified as (*E*) based on the NOE correlations between H-5 and H_3-11. Furthermore, the NOE correlation between H-8 and H_3-12 revealed the (*E*) conformation of C-6 and C-7. Therefore, compound **1** was identified as (2*E*,4*E*,6*E*,8*R*,9*S*)-9-hydroxy-2,6,8-trimethyldeca-2,4,6-trienoic acid.

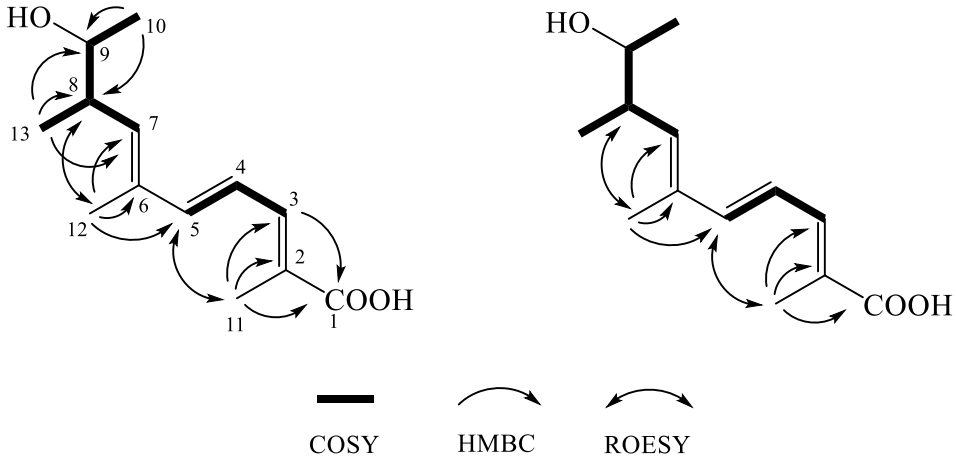

Figure 2. ^1H-^1H COSY correlations and the selected HMBC correlations of compounds **1** and **2** and the structures of compounds **1** and **2**

Compound **2** was obtained as yellow gum. The molecular formula was determined to be $C_{13}H_{20}O_3$ with four degrees of unsaturation using HR-ESI-MS (*m/z* 225.1489 $[M + H]^+$, calcd. 225.1485). The ^1H and ^{13}C NMR (Table 1) spectra of compound **2** showed almost same as those of **1**. Given the same molecular formula, compound **2** was deduced to be an isomer of **1**. The ^1H-1H COSY and HMBC (Figure 2) correlations further confirmed that compound **2** shared the same planar structure as that of compound **1**. The coupling constant between H-3 and H-4 was 11.0 Hz, which revealed the *cis* conformation between H-3 and H-4. Moreover, the NOE correlations between H-5 and H_3-11, and between H-8 and H_3-12 suggested that compound **2** have the same geometry conformation as that of compound **1**.

Table 1. ^1H and ^{13}C NMR data for compounds **1**and **2** (at 500 MHz in CD_3OD).

Position	**1**		**2**	
	δ_H (*J* in Hz)	δ_C in ppm	δ_H (*J* in Hz)	δ_C in ppm
1		172.1 (C)		172.0 (C)
2		127.0 (C)		126.9 (C)
3	7.26 (1H, *d*, *J* = 11.0)	140.6 (CH)	7.26 (1H, *dd*, *J* = 11.0, 0.5)	140.7 (CH)
4	6.48 (1H, *dd*, *J* = 15.0, 11.0)	123.0 (CH)	6.48 (1H, *dd*, *J* = 15.5, 11.0)	122.8 (CH)
5	6.60 (1H, *d*, *J* = 15.0)	145.9 (CH)	6.62(1H, *d*, *J* = 15.0)	146.1 (CH)
6		135.1 (C)		135.7 (C)
7	5.57 (1H, *d*, *J* = 10.0)	141.3 (CH)	5.66 (1H, *d*, *J* = 10.0)	140.9 (CH)
8	2.54 (1H, *ddq*, *J* = 10.0, 6.5, 6.5)	42.1 (CH)	2.60 (1H, *m*)	41.4 (CH)
9	3.55 (1H, *dq*, *J* = 6.5, 6.0)	72.6 (CH)	3.67 (1H, *dq*, *J* = 6.5, 6.0)	72.2 (CH)
10	1.12 (3H, *d*, *J* = 6.0)	21.4 (CH_3)	1.13 (3H, *d*, *J* = 6.5)	20.7 (CH_3)
11	1.94 (3H, *s*)	12.9 (CH_3)	1.94 (3H, *d*, *J* = 1.5)	12.7 (CH_3)
12	1.87 (3H, *d*, *J* = 1.0)	12.8 (CH_3)	1.80 (3H, *d*, *J* = 1.0)	12.8 (CH_3)
13	1.04 (3H, *d*, *J* = 6.5)	16.9 (CH_3)	1.02 (3H, *d*, *J* = 7.0)	16.9 (CH_3)

Attempts were made to determine the absolute configurations of C-8 and C-9 by Mosher's reaction, but the results obtained were not satisfactory. The optical rotations of compounds **1** and **2** were – 11.0 (*c* = 0.1, MeOH) and – 5.0 (*c* = 0.1, MeOH), indicating that they are epimers. Based on this information, the configurations of compounds **1** and **2** were assigned as shown in (Figure 1), and designated as penioxa acids A and B, respectively.

To evaluate their antifungal and antibacterial activities, penioxa acids A (**1**) and B (**2**) were tested for their antifungal and antibacterial activities against *C. albicans*, *E. coli*, and *S. aureus* using microdilution method. However, both compounds showed no inhibition of the growth of these pathogens at a concentration of 200 μg/mL.

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Supporting Information

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ORCID
Xinyu Liu: 0009-0007-1557-4110
Yifei Dong: 0009-0004-8386-2796
Xinwan Zhang: 0009-0009-2249-3495

Xinjun Zhang: 0009-0008-4793-961X
Caixia Chen: 0000-0003-4642-7053
Fuhang Song: 0000-0002-9162-3355
Xiuli Xu: 0000-0002-0237-1417

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