

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

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(Received March 23, 2023; Revised June 09, 2023, Accepted June 11, 2023)

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. © 2023 ACG Publications. All rights reserved.

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

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Two new trienoic acid derivatives

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 secalononic 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 separate 1 L flasks, each containing 150 g rice and 120 mL distilled H₂O. 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/H₂O (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/CH₂Cl₂ and CH₂Cl₂/MeOH to yield 16 fractions (*Fr.1–Fr.16*). Fraction *Fr.13* was further purified by on Sephadex LH-20 column eluted with CH₂Cl₂: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); ¹H NMR (500 MHz, CD₃OD): 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); ¹³C NMR (125 MHz, CD₃OD): δ (ppm) = 12.8 (CH₃, C-12), 12.9 (CH₃, C-11), 16.9 (CH₃, C-13), 21.4 (CH₃, 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); ¹H NMR (500 MHz, CD₃OD): 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); ¹³C NMR (125 MHz, CD₃OD): δ (ppm) = 12.7 (CH₃, C-11), 12.8 (CH₃, C-12), 16.9 (CH₃, C-13), 20.7 (CH₃, 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).

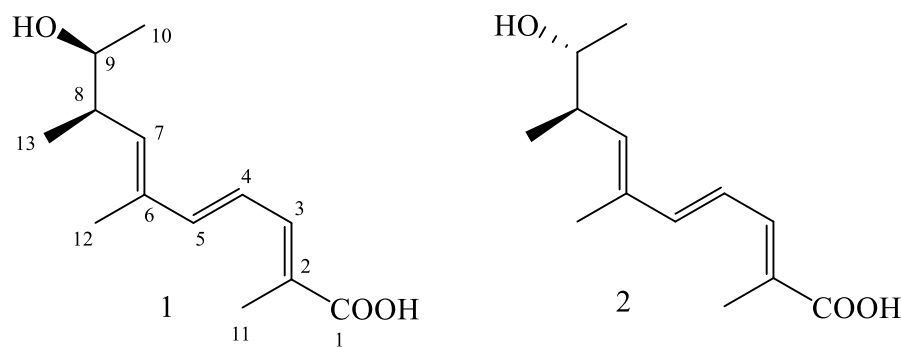


Figure 1. Structures of penioxa acids A (**1**) and B (**2**) isolated from *P. oxalicum* BTBU20213011

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, H₃-10; 1.87, *d*, $J=1.0$ Hz, H₃-12; 1.04, *d*, $J=6.5$ Hz, H₃-13), as well as one singlet methyl group (at δ_H 1.94, *s*, H₃-11). 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 H₃-10 to C-8 and C-9, and from H₃-13 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 H₃-11 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 H₃-12 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 H₃-11. Furthermore, the NOE correlation between H-8 and H₃-12 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.

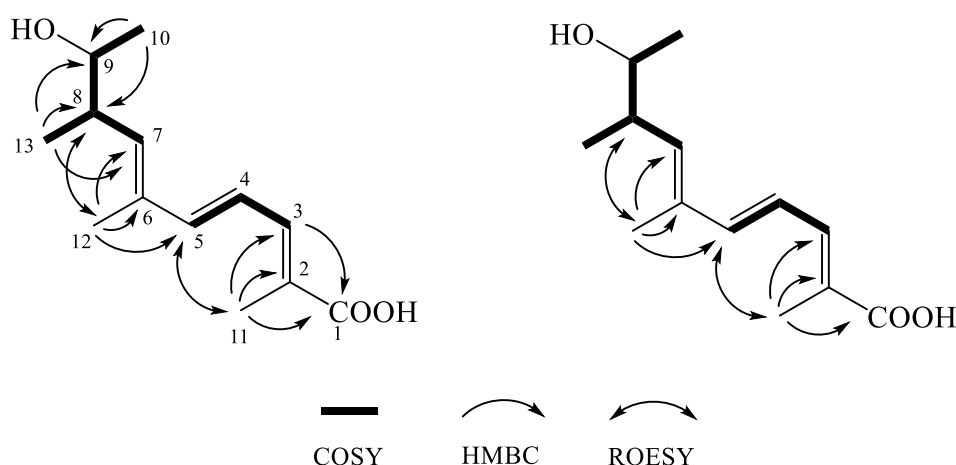


Figure 2. 1H - 1H COSY correlations and the selected HMBC correlations of compounds **1** and **2** and the structures of compounds **1** and **2**

Two new trienoic acid derivatives

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 H₃-11, and between H-8 and H₃-12 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, <i>d</i> , $J = 11.0$)	140.6 (CH)	7.26 (1H, <i>dd</i> , $J = 11.0, 0.5$)	140.7 (CH)
4	6.48 (1H, <i>dd</i> , $J = 15.0, 11.0$)	123.0 (CH)	6.48 (1H, <i>dd</i> , $J = 15.5, 11.0$)	122.8 (CH)
5	6.60 (1H, <i>d</i> , $J = 15.0$)	145.9 (CH)	6.62 (1H, <i>d</i> , $J = 15.0$)	146.1 (CH)
6		135.1 (C)		135.7 (C)
7	5.57 (1H, <i>d</i> , $J = 10.0$)	141.3 (CH)	5.66 (1H, <i>d</i> , $J = 10.0$)	140.9 (CH)
8	2.54 (1H, <i>ddq</i> , $J = 10.0, 6.5, 6.5$)	42.1 (CH)	2.60 (1H, <i>m</i>)	41.4 (CH)
9	3.55 (1H, <i>dq</i> , $J = 6.5, 6.0$)	72.6 (CH)	3.67 (1H, <i>dq</i> , $J = 6.5, 6.0$)	72.2 (CH)
10	1.12 (3H, <i>d</i> , $J = 6.0$)	21.4 (CH ₃)	1.13 (3H, <i>d</i> , $J = 6.5$)	20.7 (CH ₃)
11	1.94 (3H, <i>s</i>)	12.9 (CH ₃)	1.94 (3H, <i>d</i> , $J = 1.5$)	12.7 (CH ₃)
12	1.87 (3H, <i>d</i> , $J = 1.0$)	12.8 (CH ₃)	1.80 (3H, <i>d</i> , $J = 1.0$)	12.8 (CH ₃)
13	1.04 (3H, <i>d</i> , $J = 6.5$)	16.9 (CH ₃)	1.02 (3H, <i>d</i> , $J = 7.0$)	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 $\mu g/mL$.

Acknowledgments

This work was supported by grants from the Science and Technology Program of Tibet Autonomous Region (XZ202101YD0013C), the National Natural Science Foundation of China (81973204, 31960013).

Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>



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