SHORT REPORT

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2-Bisabolen-1,10,11,12-tetraol, A New Bisabolane Sesquiterpene from Marine Alga-Sourced *Aspergillus taichungensis* 299 and Its Antibacterial Activity against Common Pediatric Pathogens

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Abstract: One new bisabolane sesquiterpene, 2-bisabolen-1,10,11,12-tetraol (**1**), along with three known ones, (3*R*, 6*R*, 7*S*, 10*S*)-1-bisabolen-3,10,11-triol (**2**), (3*R*, 6*S*, 7*R*)-1,10-bisaboladien-3-ol (**3**), and nor-bisabolan-1,11-diol (**4**), were acquired from the extract of *Aspergillus taichungensis* 299 isolated from the inner tissue of marine red alga *Gelidium amansii*. Their structures were determined by comprehensive analysis of 1D/2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HRESIMS) data. The antibacterial assay showed that **1** displayed inhibitory activity against the growth of three common pediatric pathogens, *Haemophilus influenzae*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*, with the MIC values of 16, 8, and 32 μg/mL, respectively.

Keywords: Bisabolane sesquiterpene; *Aspergillus taichungensis*; structure elucidation; antibacterial activity.

1. Fungal Source

Aspergillus taichungensis 299 was isolated from the inner tissue of marine red alga *Gelidium amansii* collected from the coastal zone of Yantai, China, in 2018. The fresh algae were rinsed with distilled water to remove epiphytic microorganisms and immersed in 70% ethanol for 1-2 min and 0.1% mercuric chloride for 5 min, then washed with sterile water. The sterilized algal tissues were cut into small segments and placed on the Potato Dextrose Agar (PDA) medium, and the plates were incubated at 28 °C for 7 days. The *A. taichungensis* 299 was purified by transferring fungal hyphae to fresh PDA plates. This strain was identified according to analysis of the ITS regions sequence of its rDNA, and the sequence showed 100% identity to that of *A. taichungensis* (GenBank No. OP297992). The fungus was deposited in the Suqian First Hospital, China, and its registration number was 299.

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2. Previous Studies

Marine-derived *Aspergillus* can produce abundant secondary metabolites with different chemical structures, including terpenes, alkaloids, polyketides, peptides, sterols, and others [1,2]. Most of these natural products exhibited a variety of biological activities such as cytotoxicity, antiinflammatory, antioxidant, and antimicrobial activities [3,4]. On the other hand, bisabolane-type sesquiterpenenoid is known as an important member of natural products, and over 400 bisabolanes and their derivatives have been discovered, and they were mainly isolated from terrestrial plants, such as Compositae, Zingiberaceae, Apiaceae, and Phyllanthaceae [5-8]. However, more and more bisabolane sesquiterpenoids were excavated from marine-derived fungi, especially from *Aspergillus*, *Trichoderma*, and *Penicillium* [9]. In our previous study on marine-derived *Aspergillus*, a new prenylated indole alkaloid with cytotoxicity had been obtained from *A. taichungensis* 299 [10].

3. Present Study

During our ongoing investigation toward chemistry diversity of marine-derived *A. taichungensis* 299, one new bisabolane sesquiterpene, 2-bisabolen-1,10,11,12-tetraol (**1**), along with three known ones, (3*R*, 6*R*, 7*S*, 10*S*)-1-bisabolen-3,10,11-triol (**2**) [11], (3*R*, 6*S*, 7*R*)-1,10-bisaboladien-3-ol (**3**) [12], and nor-bisabolan-1,11-diol (**4**) [13], were isolated and identified from *A. taichungensis* 299. Herein, the details of isolation, structure elucidation, and antibacterial activity evaluation of compound **1** are described.

The mass cultures of *A. taichungensis* 299 and preliminary separation of its crude extract were described previously [10]. Fraction E eluted with petroleum ether (PE)/ethyl acetate (EtOAc) 1:1 was further purified by reversed-phase C18 column chromatography (CC) by a mixture of MeOH/H_2O system (from 10% to 100%, v/v) to obtain six subfractions (Fractions E1–E6). Fraction E3 (MeOH/H_2O, 1:1) was purified by silica gel CC (PE/EtOAc, 1:1) and Sephadex LH-20 (MeOH) to obtain **2** (3.8 mg) and **4** (4.2 mg). Fraction E4 (MeOH/H_2O, 7:3) was purified by silica gel CC (PE/EtOAc, 1:1) and preparative TLC (CH_2Cl_2/MeOH, 20:1) to acquire **3** (3.0 mg). Fraction G eluted with CH_2Cl_2/MeOH 20:1 was further purified by CC on reversed-phase C18 by a mixture of MeOH/H_2O system (from 20% to 100%, v/v) to obtain five subfractions (Fractions F1 – F5). Fraction F3 (MeOH/H_2O, 3:2) was purified by silica gel CC (PE/EtOAc, 1:1), preparative TLC (CH_2Cl_2/MeOH, 20:1), and Sephadex LH-20 (MeOH) to yield **1** (2.6 mg).

*2-Bisabolen-1,10,11,12-tetraol (**1**)*: Colorless oil; $[\alpha]_D^{20} = -5.6$ (*c* = 0.04, MeOH);^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HRESIMS: *m/z* 271.1918 $[M - H]^-$ (calcd for $C_{15}H_{27}O_4$, 271.1909).

Antibacterial Assay: Compound **1** was tested for antibacterial activity against four common pediatric pathogens, *Escherichia coli*, *Haemophilus influenzae*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*, by the microdilution method in 96-well plate as reported method [14]. The bacteria were cultivated and diluted to 1.5×10^8 CFU/mL in the Mueller-Hinton broth medium at 37 °C. 5 μL of the sample solution of **1** (dissolved in DMSO) and 95 μL of prepared bacteria suspension were added into the 96-well plates (the final sample concentrations were 64, 32, 16, 8, 4, 2, 1, 0.5 μg/mL) and incubated at 37 °C for 24 h. The optical density was determined at 600 nm by a multi-detection microplate reader. The positive control and negative control were chloramphenicol and DMSO, respectively.

Compound **1** was obtained as colourless oil and was given a molecular formula $C_{15}H_{28}O_4$ by analysis of HRESIMS data, consistent with two degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) displayed two methyl singlets, one methyl doublet, one doublet (δ_H 3.98, H-1) and one double doublet (δ_H 3.48, H-10) attributable to two oxygenated methines, two doublets (δ_H 3.52, H-12a, δ_H 3.46, H-12b) assignable to one oxymethylene, one broad singlet (δ_H 5.36, H-2) ascribable to an

olefinic proton. The ^{13}C NMR and DEPT spectra suggested the presence of 15 carbons, including three methyls, five methylenes, five methines, and two nonprotonated carbons. COSY correlations of H_3-14/H-7/H_2-8/H_2-9/H-10 and HMBC correlations from H_2-12 and H_3-13 to C-10 and C-11 confirmed the structure of the side chain (Figure 2). COSY correlations of H-2/H-1/H-6/H_2-5/H_2-4 and HMBC correlations from H_3-15 to C-2, C-3, and C-4 verified the structure of 3-methylcyclohex-2-en-1-ol (Figure 2). The side chain was located at C-6 via C-7 ascertained by the COSY correlation of H-6 with H-7 and the HMBC correlation from H_3-14 to C-6 (Figure 2). The relative configurations of C-1, C-6, and C-7 were inferred by comparison of NMR data with those of literature [12], which was also supported by NOESY correlations of H-1 with H-5b, H-7 and H_3-14 and of H-5b with H_3-14 (Figure 2).

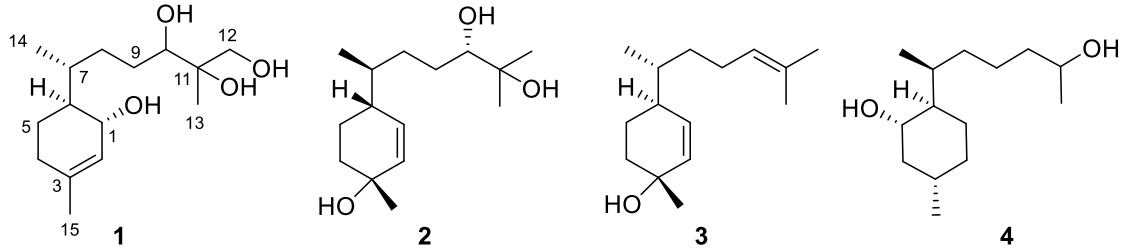

Figure 1. Chemical structures of isolated compounds **1-4**

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1** (δ in ppm) in CD_3OD

No	**δ_H (*J* in Hz)**	**δ_C, type**
1	3.98, d (8.5)	69.6, CH
2	5.36, brs	127.3, CH
3		137.5, C
4a	1.97, m	31.4, CH_2
4b	1.92, m	
5a	1.67, m	22.1, CH_2
5b	1.28, m	
6	1.37, m	47.4, CH
7	1.95, m	32.3, CH
8a	1.60, m	33.5, CH_2
8b	1.32, m	
9a	1.59, m	30.3, CH_2
9b	1.40, m	
10	3.48, dd (10.2, 1.5)	76.6, CH
11		75.7, C
12a	3.52, d (11.1)	68.3, CH_2
12b	3.46, d (11.0)	
13	1.11, s	20.5, CH_3
14	0.83, d (6.9)	14.5, CH_3
15	1.67, s	23.2, CH_3

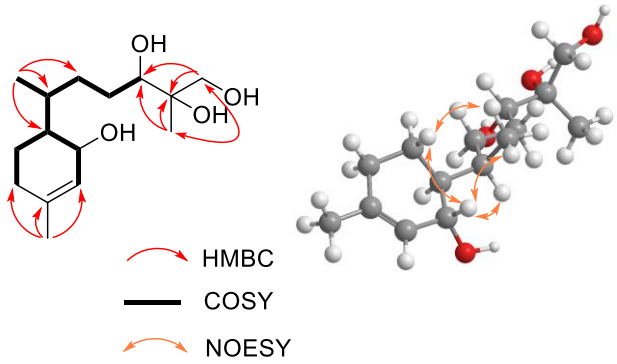

Figure 2. Key ^{1}H-^{1}H-COSY, HMBC, and NOESY correlations of **1**

2-Bisabolen-1,10,11,12-tetraol (**1**) was assayed for antibacterial effect against *E. coli*, *H. influenzae*, *S. agalactiae*, and *S. pneumoniae*. The results (Table 2) showed that **1** displayed promising antibacterial activity against *H. influenzae*, *S. agalactiae*, and *S. pneumoniae* with the MIC values ranging from 8.0 μg/mL to 32 μg/mL. However, **1** exhibited no activities against *E. coli* at 64 μg/mL.

Table 2. Antibacterial activity of compound **1** (MIC, μg/mL)

Compounds	*E. coli*	*H. influenzae*	*S. agalactiae*	*S. pneumoniae*
1	– [a]	16	8.0	32
chloramphenicol	2.0	1.0	1.0	2.0

[a] MIC > 64 μg/mL

In conclusion, our chemical investigation of the marine-derived fungus *A. taichungensis* 299 resulted in the isolation of one new bisabolane sesquiterpenoid, 2-bisabolen-1,10,11,12-tetraol (**1**), along with three known ones, (3*R*, 6*R*, 7*S*, 10*S*)-1-bisabolen-3,10,11-triol (**2**), (3*R*, 6*S*, 7*R*)-1,10-bisaboladien-3-ol (**3**), and nor-bisabolan-1,11-diol (**4**). Compound **1** was evaluated for antibacterial against four human common pediatric pathogens and showed promising antibacterial activity against *H. influenzae*, *S. agalactiae*, and *S. pneumoniae*. The antibacterial activity mechanism will be deeply inspected in the future in order to assess its potential application in the development of antibacterial agent.

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Supporting Information

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