SHORT REPORT

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A New Phenolic Bisabolane Sesquiterpenoid from the Fungus *Aspergillus* sp. Hvtc-2021zx1

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Abstract: The strain *Aspergillus* sp. Hvtc-2021zx1 was obtained and solid-state fermentation was carried out using rice medium. The medium was extracted with ethyl acetateto obtain a crude extract, which was separated by various chromatographic techniques to afford the new bisabolane **1** and four known compounds **2**–**5**. The structures were assigned by ^{1}H NMR, ^{13}C NMR, 2DNMR and ESIMS data. It should be noted that an O-methyl group at C-7 as in compound **1** was rarely found in the phenolic bisabolane skeleton. The four known compounds **2**–**5** were identified to be 11,12-dihydroxysydonicacid (**2**), sydowic acid (**3**), cyclo(D-phenylalanyl-L-leucyl) (**4**), and neoechinulin A (**5**), respectively. The NMR data of **5** was first reported in DMSO-d_6. Compound **5** was active against *S. aureus* with an MIC value of 64 μg/mL.

Keywords: Phenolic bisabolanes; *Aspergillus* sp.

1. Microorganism Material

The fungal strain Hvtc-2021zx1 was isolated from the seawater obtained in Hangzhou Bay and was identified as *Aspergillus* sp. Hvtc-2021zx1 by comparing the ITS sequence with nucleotide data stored in the GenBank database by using the BLAST method. The ITS sequence is consistent with that of *Aspergillus* sp. (MW450868.1). The spores preserved in 20% glycerol were deposited in Hangzhou Vocational & Technical College.

2. Previous Studies

In recent years, the *Aspergillus* strains have been widely studied and become a research focus of natural product chemists. Recent chemistry studies of *Aspergillus* strains led to the discovery of a great many new compounds, including indole diterpene glycosides[1], highly conjugated compound (asperaldehyde) [2], nitro-containing phenylpropionic acid derivatives [3], phthalide derivatives [4], cytotoxic nitrobenzoyl sesquiterpenoids[5], diketopiperazine heterodimers [6], sulfur-containing phenolic compounds [7], phenolic bisabolane sesquiterpenes [8], antibacterial drimane sesquiterpenes [9], isopimarane diterpenes [10], cyclic peptides [11, 12], and alkaloids [13, 14].

In our study, the strain Hvtc-2021zx1 was isolated, it was cultivated on rice solid medium and extracted with ethyl acetate (EtOAc) to give an extract. A new phenolic bisabolane and four known compounds were identified by various chromatographic separation of the extract (Figure 1). In this paper, the isolation and structural elucidation of these metabolites were expounded.

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3. Present Study

The fermentation was conducted in 20 erlenmeyer flasks (1000 mL) with 120 g of rice and 150 mL of distilled water. The contents were treated with high-pressure steam sterilization. After cooling to room temperature (r.t.), every flask was inoculated with 2.0 mL of the spore inoculum and cultured at r.t. for 25 days.

The fermented materials were extracted twice with 3000 mL of EtOAc to give an extract (6 g). The extract (9.0 g) was separated on an ODS column (MeOH/H_2O = 30:70 to 100:0) to give seven fractions (I– VII). Fraction III (0.6 g) was split by ODS column with MeOH/H_2O (30:70→60:40) as eluent to afford six fractions (IIIa-IIIf). IIIb (106 mg) was further purified on HPLC column to give **1** and **2**. Fraction IV (0.7 g) was separated by ODS using MeOH/H_2O (30:70→100:0) as mobile phase to give four subfractions IVa–IVd. Fraction IVc (152 mg) was purified by HPLC using MeCN/H_2O = 31:69 (2 mL/min) to yield **3**. Fraction IVd (121 mg) was separated on a HPLC column with MeOH/H_2O (49:51, 2 mL/min) as mobile phase to afford **4** and **5**.

*7-O-methyl-11,12-dihydroxysydonic acid (**1**)*: Colorless oil, $[\alpha]^{25}_D$ 0 (c = 0.2, MeOH); UV (MeOH) λ_{max} 220 (4.90), 246 (4.01) nm. ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z*: 311.1510 $[M - H]^-$ (calcd for $C_{16}H_{23}O_6^-$, 311.1500).

*Cyclo(D-phenylalanyl-L-leucyl) (**4**)*: δ_C 21.3, 23.4, 24.6, 40.2, 45.2, 54.1, 57.4, 128.5, 129.6, 129.6, 131.8, 131.8, 136.7, 168.9, 170.6.

*Neoechinulin A (**5**)*: δ_C 19.6, 27.5, 39.0, 50.5, 103.4, 110.2, 111.6, 111.6, 118.9, 119.4, 120.7, 124.9, 126.0, 135.1, 144.0, 145.2, 159.9, 166.0.

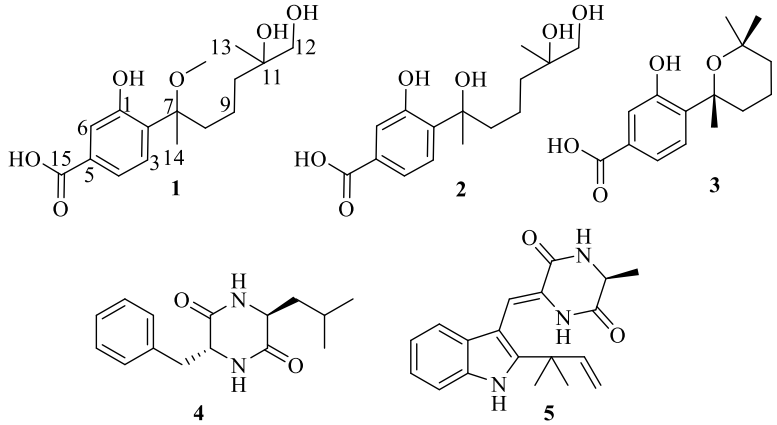

Figure 1. Metabolites from *Aspergillus* sp. Hvtc-2021zx1

Compound **1** was obtained as a colorless oil, the molecular formula of **1** was designated to be $C_{16}H_{24}O_6$ according to the ion peak at *m/z* 311.1510 $[M - H]^-$ ($C_{16}H_{23}O_6$, calcd. 311.1500) in the HRESIMS spectra. The ^{1}H NMR spectrum showed the signals for a methoxy group [δ_H 3.24 (3H, s)], two tertiary methyl groups [δ_H 1.64 (3H, s), 1.06 (3H, s)], three aromatic protons [δ_H 7.47 (1H, d, *J* = 8.2, 1.5 Hz), 7.39 (1H, d, *J* = 1.5 Hz, H-5), 7.25 (1H, d, *J* = 8.2 Hz) for a 1,2,4-trisubstituted benzene moiety, an oxymethylene (δ_H 3.29), and six methylene protons (δ_H 1.90, 1.40, 1.29).

The ^{13}C NMR data exhibited 16 carbons, consisting with the molecular formula. The carbon resonances could be assigned to be a carbonyl carbon (δ_C 170.8), six aromatic carbons including three protonated carbons (δ_C 128.9, 121.7, 118.6) and three non-protonated carbons (δ_C 156.8, 134.6, 133.6),

four methylene carbons (δ_C 70.3, 41.0, 39.6, 19.1) including one oxygenated, three methyl carbons (δ_C 50.7, 23.6, 22.7), and two oxygenated non-protonated carbons (δ_C 83.3, 73.6), according to the HSQC spectrum.

The aforementioned data were almost the same as those of a co-isolated known analog 11,12-dihydroxysydonic acid (**2**) [15], which indicated that compounds **1** and **2** were structurally related bisabolanes. A detailed comparison of their ^{1}H and ^{13}C NMR data revealed that the only difference between 1 and **2** was owing to the presence of the signals for a methoxy (δ_H 3.24; δ_C 50.7) in **1**, which indicated that **1** was 7-O-methyl derivative of **2**. The methoxy group was located at C-7 by the HMBC correlation from the methoxy protons at δ_H 3.24 to the oxygenated non-protonated carbon at 83.3 ppm (C-7). The structure of **1** was thus determined as in figure 1 and was further confirmed by detailed 2D NMR analyses (Figure 2).

The electronic circular dichroism spectrum displayed negligible Cotton Effect, and the optical rotation was found to be almost zero, indicating **1** to be a racemic mixture. Further chiral resolution of compound **1** on a Daicel chiralpack AD-H chiral column was failed. Thus, compound **1** was reported in racemic form in the current study. Compound **1** was named (±)-7-O-methyl-11,12-dihydroxysydonic acid.

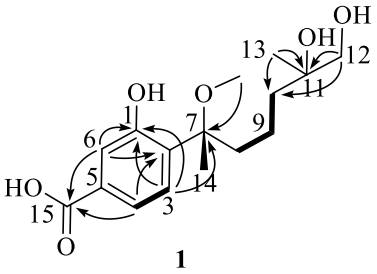

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Figure 2. Key HMBC (⟶) and ^{1}H-^{1}H COSY (▬) correlations of **1**.

Table 1. NMR Data for **1** in methanol-d_4 (^{1}H NMR in 400MHz, ^{13}C NMR in 100 MHz)

position	1		2
	δ_C, type	δ_H (*J* in Hz)	δ_c
1	156.8, C		157.1, C
2	134.6, C		138.2, C
3	128.9, CH	7.25, d (8.2)	128.1, CH
4	121.7, CH	7.47, dd (8.2, 1.5)	121.8, CH
5	133.6, C		132.3, C
6	118.6, CH	7.39, d (1.5)	118.9, CH
7	83.3, C		78.1, C
8	41.0, CH_2	1.90, m	44.3, CH_2
9	19.1, CH_2	1.40, m; 1.29, m	19.5, CH_2
10	39.6, CH_2	1.40, m	40.0, CH_2
11	73.6, C		74.0, C
12	70.3, CH_2	3.29, s	70.6, CH_2
13	23.6, CH_3	1.06, s	24.0, CH_3
14	22.7, CH_3	1.64, s	29.2, CH_3
15	170.8, C		170.5, C
-OCH_3	50.7, C	3.24, s	

The known compounds **2**–**5** were determined to be 11,12-dihydroxysydonicacid (**2**) [15], sydowic acid (**3**) [16], cyclo(*D*-phenylalanyl-*L*-leucyl) (**4**) [17], and neoechinulin A (**5**) [18] through comparisons of the ^{1}H NMR or ^{13}C NMR data with the published data in the literature.

A new phenolic bisabolane sesquiterpenoid

Compounds **1**–**5** were screened for the antibacterial activity toward *Staphylococcus aureus* and *Escherichia coli*, only **5** showed moderated effect against *S. aureus* with an MIC value of 64 μg/mL, the other compounds showed negligible antibacterial activity at the concentration of 256 μg/mL.

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Supporting Information

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