SHORT REPORT

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Cytotoxic Drimane-type Sesquiterpenoids from the Fungus *Aspergillus flavipes* 297

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Abstract: Chemical investigation of the marine-derived fungus *Aspergillus flavipes* 297 led to the isolation and identification of three drimane-type sesquiterpenoids, asperflavinoid A (**1**) and (6-strobilactone-B) esters of (*E*,*E*)-6,7-dihydroxy-2,4-octadienoic acids (**2** and **3**). Asperflavinoid A (**1**) was characterized as unseparated diastereomers and its chemical structure was determined by spectroscopic analysis of NMR and MS data. In the cytotoxic assay, compound **1** showed promising inhibitory effects on HepG2 and MKN-45 cell lines.

Keywords: *Aspergillus flavipes*; natural products; drimane sesquiterpenoids; cytotoxicity.

1. Plant Source

The fungus 297 was isolated from the fresh seawater that was collected at coastal zone of Yantai, China, in October 2019. This fungal isolate was identified as *Aspergillus flavipes* according to the comparison of the ITS region of the rDNA sequence. The ITS sequence has been submitted to the GenBank database (http://www.ncbi.nlm.nih.gov) with the accession number of MZ669896. This strain was deposited in the First Affiliated Hospital of Soochow University with a voucher specimen of 297.

2. Previous Studies

Filamentous fungi especially those belonging to the genus *Aspergillus* have proven to be a treasure producer of structurally diverse secondary metabolites with attractive bioactivities [1]. Moreover, fungi residing in marine ecological environment suffered from high salinity, hypoxia stress, and extreme temperature, which evolved novel metabolic pathways to synthesize massive specialised metabolites [2]. As widely-observed species, marine-derived *A. flavipes* has reported to produce

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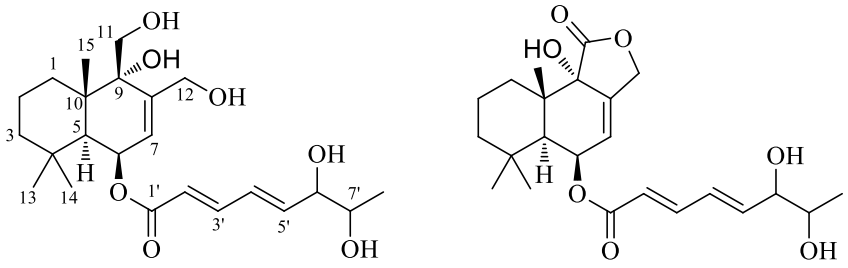
numerous metabolites with diverse chemical skeletons, including cyclopeptides [3], butenolides [4], phenylbutyrolactones [5], cerebrosides [6], and cytochalasans [7]. It is estimated that more than 60 compounds were isolated and identified from *A. flavipes*. The dominant structural types are butenolides and butyrolactones, indicating these compounds may be active pharmaceutical ingredients of this fungus. Moreover, many reported metabolites showed pronounced bioactivities, such as α-glucosidase inhibitory, antibacterial, antibiofilm, cytotoxic, and anti-inflammatory effects. It should be pointed out that many reports revealed that *A. flavipes* was found to possess anti-inflammatory activity and thus it can be further explored in the therapeutic area [8].

3. Present Study

The solid-state fermentation of this fungal strain was performed using rice medium (rice 100 g and fresh filtered seawater 100 mL) at room temperature for 30 days. The fungus formed mycelium through vegetative growth stage. Then the fermented materials were extracted with EtOAc for three times (each with 100 mL per flask). The organic phase was collected and concentrated to afford a crude extract (ca. 9.8 g). The obtained crude extract was subjected to open silica gel column chromatography with a mixed CH_2Cl_2–MeOH of increasing polarities (from 50:1 to 10:1, v/v). A total of five fractions (Fr. 1–Fr. 5) were generated. Fr. 5 (1.0 g), eluted with CH_2Cl_2–MeOH 10:1, was separated by preparative thin-layer chromatography (organic solvents: CH_2Cl_2–MeOH, 20:1), and then by Sephadex LH-20 (MeOH) to afford compounds **1** (12.6 mg) and **2**–**3** (5.8 mg) (Figure 1).

*Asperflavinoid A (**1**):* Colorless oil; UV (MeOH): λ_{max} (log ε): 286 (3.38) nm; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; positive HREIMS: *m/z* 447.2360 $[M + Na]^+$ (calculated for $C_{23}H_{36}O_7Na$, 447.2359).

Cytotoxic assay: Cytotoxic activities of the isolated compounds **1**–**3** against A549, HepG2, HCT-116, and MKN-45 human tumor cells were assessed by the CCK-8 method [9]. Commercial adriamycin was used as the positive control.



1 **2** and **3**

Figure 1. Chemical structures of compounds **1**–**3**

Asperflavinoid A (**1**) was isolated as an oily mixture. A molecular formula of $C_{23}H_{36}O_7$ was assigned based on the positive-mode HRESIMS data at *m/z* 447.2360 $[M + Na]^+$ (calculated for $C_{23}H_{36}O_7Na$, 447.2359). In the ^{1}H NMR spectrum, eight oxygenated/olefinic protons [δ_H 7.16 (1H, m), 6.40 (1H, m), 6.29 (1H, m), 5.89 (1H, d, *J* = 15.3 Hz), 5.75 (1H, d, *J* = 4.8 Hz), 5.52 (1H, br s), 3.82 (1H, dd, *J* = 12.0, 5.4 Hz), and 3.47 (1H, m)], two oxygenated methylene groups [δ_H 4.12 (2H, d, *J* = 4.1 Hz), 3.49 (1H, m), and 3.43 (1H, m)], and four methyls [δ_H 1.15 (3H, s), 1.03 (3H, s), 1.01 (3H, d, *J* = 6.3 Hz), and 0.89 (3H, s)] were observed (Table 1). Five additional protons that were attributable to exchangeable protons at δ_H 4.97 (1H, d, *J* = 5.0 Hz), 4.82 (1H, t, *J* = 5.0 Hz), 4.61 (1H, br s), 4.57 (1H, br s), and 4.38 (1H, s) were also detected. The ^{13}C NMR spectrum together with DEPT spectra indicated the presences of 23 carbon resonances, including four methyls (δ_C 33.1, 25.0, 19.7, and 19.2), five methylenes (with two oxymethylenes at δ_C 62.1 and 61.0), nine methines (with five olefinic at δ_C

146.0, 145.0, 127.7, 121.1, and 120.3, and three oxygenated at δ_C 75.4, 70.0, and 66.7), and five unprotonated carbons (with one ester carbonyl at δ_C 166.1) (Table 1).

Table 1. ^{1}H and ^{13}C NMR data for compound **1** (in DMSO-d_6, δ in ppm)

No.	δ_H (mult., *J* in Hz)	δ_C, type	No.	δ_H (mult., *J* in Hz)	δ_C, type
1	1.84, t (15.1) 1.41, m	32.2, CH_2	15	1.15, s	19.2, CH_3
2	1.58, dd (13.3) 1.41, m	18.6, CH_2	1'		166.1, C
3	1.27, d (12.3) 1.11, m	44.5, CH_2	2'	5.89, d (15.3)	121.1, CH
4		33.7, C	3'	7.16, dd (15.3, 11.2)	145.0, CH
5	1.95, d (3.9)	45.4, CH	4'	6.40, m	127.7, CH
6	5.52, br s	66.7, CH	5'	6.29, m	146.0, CH
7	5.75, d (4.8)	120.3, CH	6'	3.82, dd (12.0, 5.4)	75.4, CH
8		145.1, C	7'	3.47, m	70.0, CH
9		74.5, C	8'	1.01, d (6.3)	19.7, CH_3
10		40.6, C	9-OH	4.38, s	
11	3.49, m 3.43, m	62.1, CH_2	11-OH	4.61, br s	
12	4.12, d (4.1)	61.0, CH_2	12-OH	4.82, t (5.0)	
13	0.89, s	33.1, CH_3	6'-OH	4.97, d (5.0)	
14	1.03, s	25.0, CH_3	7'-OH	4.57, br s	

*^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz)

^{1}H-^{1}H COSY correlations between H-2'/H-3'/H-4', H-4'/H-5'/H-6', H-6'/H-7'/H-8', H-6'/6'-OH, and H-7'/7'-OH established a fatty acyl moiety (from H-2' to H-8') with two hydroxyls substituted at C-6' and C-7' (Figure 2A). The key HMBC correlations shown in Figure 2A indicated the presence of a drimane sesquiterpenoid skeleton. The fatty acyl moiety was linked to C-6 of drimane core framework through an ester carbonyl (C-1') as supported by HMBC correlations from H-2' to C-1' and H-6 to C-1'. The NOE correlations between H-5/H-14, H-6/H-14, H-13/H-15, and H-11/H-15 (Figure 2B) determined the relative configurations, which were deduced to be the same as those of previously reported analogues [10,11].

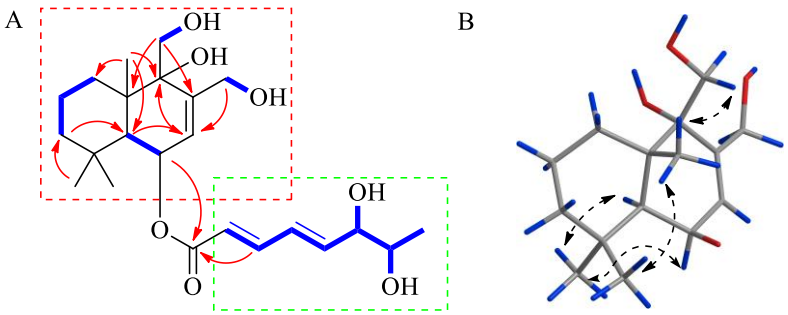


Figure 2. (A) Key ^{1}H-^{1}H COSY (blue bold lines) and HMBC (red arrows) correlations for **1**; (B) Key NOE (black dashed arrows) correlations for the drimane core framework of **1**

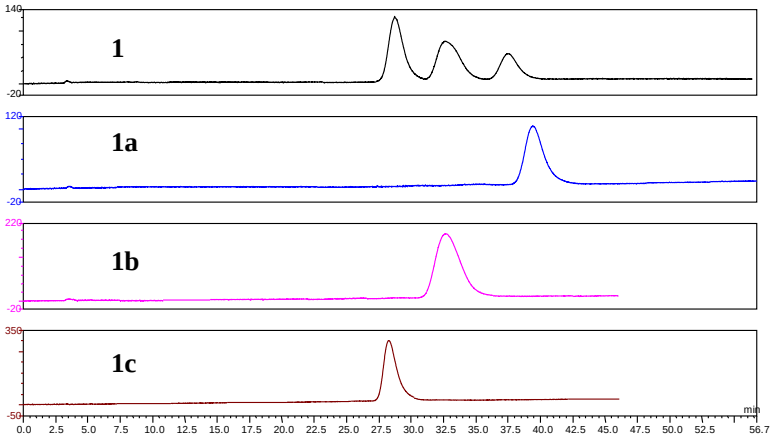


Figure 3. Chiral HPLC separation of **1** (Whelk-O1 rpirkle-cencept chiral HPLC column, eluted with *n*-hexane−ethanol 9:1, 1.0 mL/min)

The chemical structure of asperflavinoid A (**1**) could be found in SciFinder. However, there were no references, as well as NMR data searched. In addition, asperflavinoid A (**1**) was considered to be diastereomers at C-6' and C-7' based on their barely discernible deviation of the ^{13}C NMR data near both positions. **1** was subjected to chiral HPLC separation. Three enantiomers **1a**, **1b**, and **1c** were successfully obtained (Figure 3). However, they could change into a mixed state as soon as possible, indicating that **1** is unseparated diastereomers. This was also proved by reported analogues, (6-strobilactone-B) esters of (*E*,*E*)-6,7-epoxy-2,4-octadienoic acids [10] and (6-strobilactone-B) esters of (*E*,*E*)-6,7-dihydroxy-2,4-octadienoic acids (**2** and **3**) [11]. Accordingly, based on above discussion, the stereochemistry of C-6' and C-7' were unsolved.

The effects of the isolated compounds **1**−**3** against A549, HepG2, HCT-116, and MKN-45 cell lines were studied (Table 2). **1** exhibited mild cytotoxic activity against HepG2 and MKN-45 cells, with the IC_{50} values of 38.5 and 26.8 μg/mL, respectively.

Table 2. Cytotoxicity of isolated compounds against human cancer cell lines[a]

Compounds	A549	HepG2	HCT-116	MKN-45
1	> 50	38.5 ± 2.0	> 50	26.8 ± 1.7
2 and **3**	> 50	> 50	> 50	> 50
Adriamycin[b]	2.5 ± 0.2	1.8 ± 0.1	3.8 ± 0.3	6.2 ± 0.3

[a] IC_{50} values, μg/mL, mean ± SD. [b] Positive control.

The fungus *A. flavipes* are known to be a promising producer of bioactive metabolites. This study reported the isolation of three drimane-type sesquiterpenoids, asperflavinoid A (**1**) and (6-strobilactone-B) esters of (*E*,*E*)-6,7-dihydroxy-2,4-octadienoic acids (**2** and **3**), as well as their cytotoxicity against four human cancer cell lines. Interestingly, the new compound **1** was unseparated diastereomers **1a**, **1b**, and **1c**. The study limitations are i) the mechanism of forming diastereomers is uninvestigated and ii) the configurations of C-6' and C-7' remain unsolved. Further study should particularly be focused on the process of these diastereomers and fully determination of stereochemistry. Moreover, compound **1** was found to be toxic towards HepG2 and MKN-45 cells, indicating its potential as antitumor lead compound. More in-depth studies on possible mechanisms and pathways of this fungus and obtained compounds should be performed in the future.

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Supporting Information

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

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