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Asperaldehyde, A New Conjugated Compound from the Marine-Derived Fungus *Aspergillus* sp. LPFH-6

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Abstract: The fungal strain *Aspergillus* sp. LPFH-6 was cultured on solid rice medium with the addition of artificial salt. The culture medium was extracted with the solvent EtOAc to afford an extract, which was separated by various chromatographic techniques to give 8 compounds (**1**–**8**). The structures were determined by extensive analyses of the spectroscopic data including 1D (^{1}H and ^{13}C NMR), 2D NMR (^{1}H-^{1}H COSY, HSQC, HMBC, NOESY), and the MS data. Compound **1** was identified to be a highly conjugated compound that contained a rare 5-(2-methoxyphenyl)penta-2,4-dienal moiety. The known compounds were identified as yaminterritrem B (**2**), butyrolactone I (**3**), butyrolactone V (**4**), sulochrin (**5**), monomethylsulochrin (**6**), questinol (**7**), and 7-hydroxyemodin (**8**). Bioassay showed that compounds **2**–**4** and **8** displayed better α-glucosidase inhibitory activity than the positive control acarbose with IC_{50} values of 0.25, 0.09, 0.12, and 0.27 mM, respectively.

Keywords: *Aspergillus* sp.; marine-derived fungus; asperaldehyde. © 2023 ACG Publications. All rights reserved.

1. Introduction

Fungal strains derived from marine environment have been proven to produce a series of structurally unusual molecules bearing broad-ranging pharmacological activity. In the past ten years, marine-derived fungi have been recognized to be a rich and potential source to develop drug lead compound. According to the statistics, nearly half of the marine-derived new compounds were from fungal strains in the past five years [1-3].

The *Aspergillus* strains were very common in nature, consisting of over 300 species, several familiar members are as follows: *Aspergillus fumigatus*, *Aspergillus versicolor*, *Aspergillus terreus*, *Aspergillus niger*. Marine-derived *Aspergillus* species have been found to be an outstanding source of structurally diverse compounds with various pharmacological properties.

Recent chemical studies of marine *Aspergillus* strains led to the identification of new cytotoxic ergostane-type sterols containing a rare unsaturated side chain (aspersterols A–D) [4], novel alkaloids such as the notoamide-type bearing significant cytotoxic activities (sclerotiamides C–H) [5, 6], new terpenoids including meroterpenoids (aspermeroterpenes D and E), sesterterpenoids (two ophiobolin P derivatives), and sesquiterpenoids (asperflavinoid A, aspterrics A and B) [7-10], bioactive polyketides such as the cytotoxic globoscin derivative fischerin B [11, 12], and the cyclohexapeptides

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petrosamides A–C with significant pancreatic lipase inhibitory activity [13].

In our search for new metabolites from marine fungi, a highly conjugated compound **1** and seven known compounds (**2**–**8**) were isolated from the fungal strain *Aspergillus* sp. LPFH-6. In this paper, the isolation, structure identification, and inhibitions on NO production and α-glucosidase of these metabolites were stated.

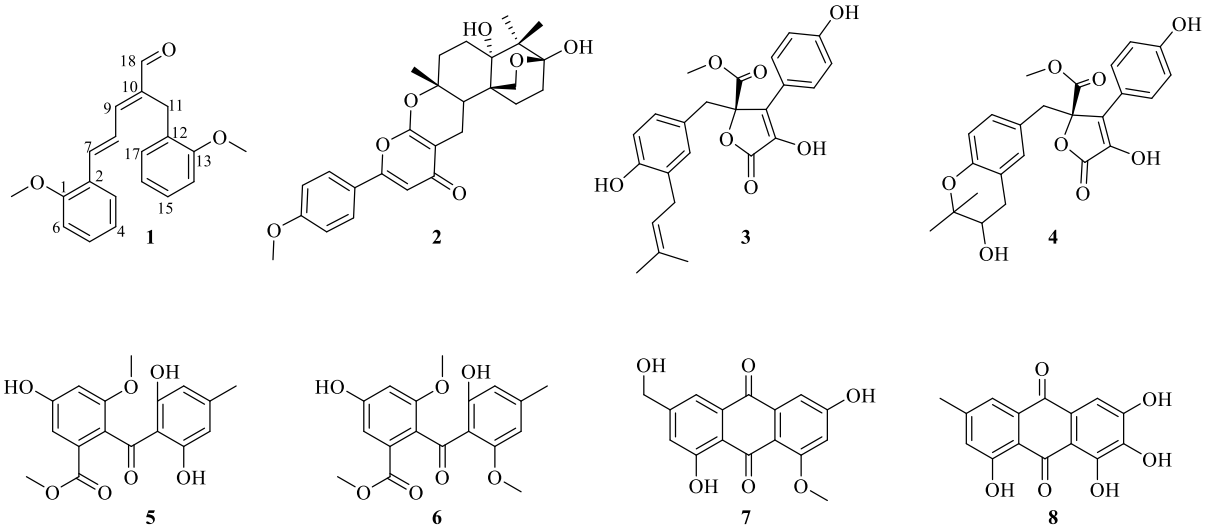

Figure 1. Compounds **1**–**8** from the marine-derived fungus *Aspergillus* sp. LPFH-6

2. Materials and Methods

2.1. General Experimental Procedures

UV spectrum was recorded on a Cary 300 spectrometer. The ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance-400FT NMR spectrometer. HRESIMS spectrum was achieved on a Waters Xevo G2 Q-TOF spectrometer equipped with an ESI source. Semi-preparative high-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-6AD pump with a UV detector, and a YMC-Pack ODS-A column was used for separation.

2.2. Microorganism Material

The fungal strain LPFH-6, isolated from sea sediments obtained from the Hangzhou Bay, was identified to be *Aspergillus* sp. based on morphological features and by comparison of the ITS region of the rDNA sequence with those recorded in GenBank. The sequenced data have been deposited in GenBank (http://www.ncbi.nlm.nih.gov) with the accession number OQ254751. The strain was kept in store in the First People's Hospital of Linping District of Hangzhou.

2.3. Fermentation and Isolation

The fermentation was carried out in 25 fernbach flasks (500 mL), 80 g of rice and 90 mL of artificial seawater were added. The contents were soaked for 6 h before autoclaving in a steam sterilizer. The fresh mycelia of the target strain were grown on PDA medium at room temperature (r.t.) for 3 days and were then transferred into the flasks. The mycelia were further incubated at r.t. for 30 days.

The fermented materials were extracted using 4 L of EtOAc for three times to afford the extracting solution, which was concentrated under vacuum to afford an extract. The extract (5 g) was separated by middle chromatogram isolated gel (MCI) using MeOH/H_2O (20:80→100:0) as eluent to obtain eight fractions (F1–F8). F8 was subject to a silica gel using petroleum ether/ethyl acetate (10:1 to 2:1) to afford **1** (7.2 mg). Fraction F4 was split on an ODS silica gel CC to give five subfractions

F4a−F4e, fraction F4c was separated by HPLC equipped with a semi-preparative YMC-pack ODS-A column (*S*-5 μm, 12 nm, 250 × 12 mm) using ACN/H_2O (55:45, 3 mL/min, C18 column) to obtain **3** (16.0 mg) and **4** (5.0 mg), fraction F4e was separated by HPLC using ACN/H_2O (60:40, 3 ml/min) to give **2**. Fraction F3 was chromatographed over an ODS silica gel CC eluted with MeOH/H_2O (40:60→100:0) to afford four subfractions F3a–F3d, fraction F3a was separated by HPLC using ACN/H_2O (45:55, 3 mL/min, C18 column) to obtain **2** (5.5 mg), fraction F3c was separated by HPLC using ACN/H_2O (50:50, 3 mL/min, C18 column) to obtain **5** (3.7 mg) and **6** (3.2 mg). Fraction F2 was chromatographed by ODS silica gel CC with MeOH/H_2O (20:80 to 100:0) as eluent to obtain five subfractions F2a−F2f, fraction F2c was separated by HPLC eluted with ACN/H_2O (30:70, 3 mL/min, C18 column) to give **8** (4.4 mg). Fraction F2e was chromatographed over HPLC eluted with MeOH/H_2O (35:65) to give **7** (3.6 mg).

*Asperaldehyde (**1**)*: Yellow oil; UV (MeOH) λ_{max} (log ε) 346 (4.33), 315 (4.22), 214 (4.45); IR (KBr) ν_{max} 3030 2962, 2838, 1675, 1612, 1490, 1242, 1110, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1; HRESIMS *m*/*z* 331.1306 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_3Na^+$, 331.1305).

*Yaminterritrem B (**2**)*: Yellow powder; ^{13}C NMR (100 MHz, DMSO-d_6) 15.1, 19.2, 20.1, 21.1, 26.8, 28.2, 28.7, 33.1, 41.5, 46.0, 48.6, 55.4, 65.5, 73.2, 83.9, 97.5, 98.6, 107.0, 114.5, 122.7, 127.2, 158.2, 161.4, 161.6, 178.6.

*Butyrolactone I (**3**)*: Yellow oil; ^{13}C NMR (100 MHz, CD_3OD) 17.8, 26.0, 28.7, 39.6, 53.8, 86.8, 115.0, 116.6, 123.3, 123.6, 125.1, 128.4, 128.9, 129.8, 130.3, 132.4, 133.0, 140.0, 155.1, 159.3, 170.5, 171.7.

*Butyrolactone V (**4**)*: Yellow oil; ^{13}C NMR (100 MHz, CD_3OD) 20.9, 25.8, 32.0, 39.5, 53.9, 70.4, 77.9, 86.7, 116.6, 117.2, 120.5, 123.2, 126.1, 129.1, 130.3, 130.4, 132.9, 140.0, 153.4, 159.3, 170.4, 171.5.

*Asperterpene K (**5**):* Red oil; ^{13}C NMR (100 MHz, DMSO-d_6) 21.6, 52.1, 56.0, 103.4, 107.2, 107.6, 109.1, 126.2, 127.9, 147.4, 156.8, 158.1, 161.7, 165.7, 199.7.

*Asterrelenin (**6**)*: Red oil; ^{13}C NMR (100 MHz, CD_3OD) 22.4, 52.5, 56.2, 56.5, 104.0, 108.7, 111.4, 129.9, 159.8, 162.7, 168.0.

*Questinol (**7**)*: Red oil; ^{13}C NMR (100 MHz, DMSO-d_6) 56.2, 62.1, 105.1, 107.7, 115.2, 115.7, 120.9, 132.2, 136.8, 151.2, 161.8, 163.6, 182.6, 186.0.

*7-Hydroxyemodin (**8**)*: Red oil; ^{13}C NMR (100 MHz, DMSO-d_6) 21.6, 109.2, 109.9, 113.5, 120.3, 123.6, 124.7, 133.2, 139.1, 148.4, 151.4, 152.2, 161.3, 180.5, 190.6.

2.4 α-Glucosidase Inhibitory Assay

The *α*-glucosidase inhibitory activities were assayed according to the method reported [14].

2.5. Determination of NO Production.

The procedure to determine the inhibitory activities on NO production were according to that in the literature [15].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1**, a yellow oil, had the molecular formula $C_{20}H_{20}O_3$ as established by the HRESIMS *m/z* 331.1306 $[M + Na]^+$ (calcd 331.1305) and NMR data (Table 1), requiring 11 double bond

equivalents. The ^{1}H NMR spectrum exhibited the presences of two aromatic methoxys [(δ_H 3.89 (3H, s) and 3.87 (3H, s)], three olefinic protons [δ_H 7.40 (1H, d, *J* = 15.6 Hz), 7.50 (1H, dd, *J* = 15.6, 11.0 Hz), 7.29 (1H, d, *J* = 11.0 Hz)] including two (δ_H 7.40 and 7.50) for a *trans* double bond, two 1,2-disubstituted benzenes [δ_H 7.04 (1H, d, *J* = 8.3 Hz), 7.33 (1H, t, *J* = 8.1 Hz) , 6.96 (1H, m) , 7.61 (1H, dd, *J* = 7.7, 1.4 Hz) , 6.94 (1H, d, *J* = 8.0 Hz) , 7.15 (1H, t, *J* = 7.8 Hz) , 6.82 (1H, t, *J* = 7.4 Hz) , 7.09 (1H, d, *J* = 7.4 Hz)], a methylene [δ_H 3.78 (2H, s)], and an aldehyde proton [δ_H 9.59 (1H, s)] (Table 1).

Table 1. ^{1}H (400 Hz) and ^{13}C NMR (100 Hz) Data of **1** in Acetone-d_6 (δ in ppm)

	1	
No.	**δ_H (mult., *J* in Hz)**	**δ_C**
1		158.7, C
2		126.0, C
3	7.61, dd (7.7, 1.4)	128.3, CH
4	6.96, m	121.7, CH
5	7.33, t (8.1)	131.6, CH
6	7.04, d (8.3)	112.4, CH
7	7.40, d (15.6)	137.1, CH
8	7.50, dd (15.6, 11.0)	125.6, CH
9	7.29, d (11.0)	151.5, CH
10		140.8, C
11	3.78, s	24.4, CH_2
12		128.6, C
13		158.2, C
14	6.94, d (8.0)	111.4, CH
15	7.15, t (7.8)	128.2, CH
16	6.82, t (7.4)	121.2, CH
17	7.09, d (7.4)	130.3, CH
18	9.59, s	194.6, CH
1-OCH_3	3.89, s	56.0, CH_3
13-OCH_3	3.87, s	55.8, CH_3

The ^{13}C NMR spectrum exhibited the presences of twenty carbon resonances, which were attributed to twelve aromatic carbons for two benzenes, four olefinic carbons for two double bonds, an aldehyde carbon (δ_C 194.6), two methoxy carbons (δ_C 56.0, 55.8), and a methylene carbon (δ_C 24.4). The 11 degrees of unsaturation were completely explained by the two benzenes, two double bonds, and the aldehyde group, revealing that there was no additional ring in the structure.

The structure was established by detailed interpretation of the 2D NMR analyses (Figure 1). The COSY relationship indicated the presence of three spin systems including CH-3–CH-4–CH-5–C-6, CH-14–CH-15–CH-16–CH-17, CH-7–CH-8–CH-9. The HMBC correlations from H-3, H-6 to C-1 and C-2, and from H-14, H-17 to C-12, C-13, along with the HMBC correlations from the methoxy groups to C-1 and C-13 demonstrate the presence of two 1-methoxy-2-alkyl-benzene moieties. The HMBC correlations from H-9 to C-10, C-18 established a (2*E*,4*E*)-penta-2,4-dienal unit. Additional HMBC correlations from H_2-11 to C-9, C-10, C-18 indicated that a methylene was attached to C-10, thus assigning a (2*E*,4*E*)-penta-2-methylene-2,4-dienal unit, this fragment and the two benzene moieties were finally assembled by the HMBC correlations from H-7 to C-1, C-2, C-3, and the HMBC correlations from H_2-11 to C-12, C-13, C-17. The double bond Δ^7 was determined to have a *E*-configuration by the coupling constant of $J_{7,8}$ (15.6 Hz), and the double bond Δ^9 was assigned to have a *Z*-configuration by the NOE correlations between H-9 and H-18.

The structure of compound **1** was thus established as in figure 1, containing a rare conjugated system (5-(2-methoxyphenyl)penta-2,4-dienal moiety), whose analogs were rarely found in nature. Compound **1** was named as asperaldehyde.

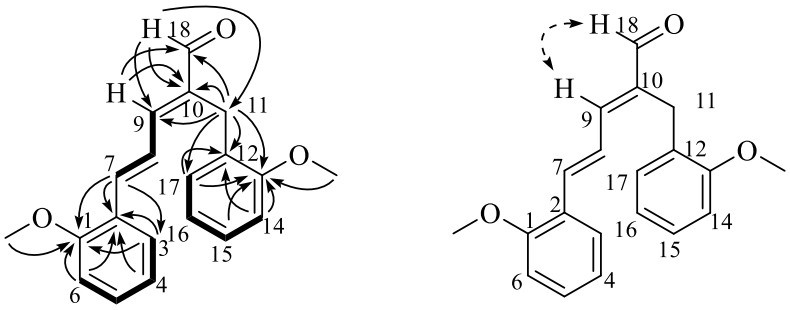

Figure 2. Key ^{1}H–^{1}H COSY (—), HMBC (⤳), and NOESY correlations (↖-↗) of **1**.

Additionally, the remaining compounds were assigned to be yaminterritrem B (**2**) [16], butyrolactone I (**3**) [17], butyrolactone V (**4**) [17], sulochrin (**5**)[18], monomethylsulochrin (**6**) [19], questinol (**7**) [20], 7-hydroxyemodin [21] based on sharing almost identical NMR data with the assigned structures reported in the literature.

*3.2. α-Glucosidase Inhibitory Effects of Compounds **1**–**8***

The inhibitions of compounds **1**–**8** on α-glucosidase were first assessed at an initial concentration of 200 μM [14]. Compounds **2**, **3**, **4**, and **8** showed inhibitions more than 30%, these four compounds were then selected for further test to determine the IC_{50} values. The results showed that compounds **2**–**4**, and **8** were more active than the positive control acarbose with IC_{50} values of 0.25, 0.09, 0.12, and 0.27 mM, respectively.

Table 2. on α-Glucosidase inhibitory effects of compounds **1**–**8**

No.	Inhibition（%）[a]	IC_{50} (mM)
1	17%	nt.[b]
2	42%	0.25
3	89%	0.09
4	74%	0.12
5	13%	nt.[b]
6	21%	nt.[b]
7	24%	nt.[b]
8	41%	0.27
Acarbose		0.31

[a] at 200 μM, [b] not tested

3.3. Inhibitory Effects Toward NO Production in LPS-Activated RAW 264.7 Macrophages

First, the cell viability was evaluated by MTT method to determine the cytotoxicity of compounds **1**–**8** on RAW 264.7 cells at an initial concentration of 50 μM. The data indicated that all compounds were non-toxic with over 90% cell survival. Then, the isolated metabolites were further tested for the inhibition toward NO production in LPS-activated RAW 264.7 macrophages at the concentration of 20 μM. As a result, only compound **3** showed weak inhibition rate of 32.7%, while other compounds exhibited inhibition rate less than 15%.

Supporting Information

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

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