

Butenolide Derivatives from the Fungus *Aspergillus terreus* and Their α -Glucosidase Inhibitory Effects

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Abstract: The strain was isolated from the sea sediment of Hangzhou Bay and was cultured on solid fermentation using rice bran. The fermented material was extracted with ethyl acetate. The chemical study of the extract led to the isolation of 8 butenolide derivatives (**1–8**). Their structures were elucidated by detailed analyses of spectroscopic techniques including 1D and 2D NMR and HRESIMS data. The absolute configuration of **1** was determined by comparing the experimental ECD curve of **1** with that of the computed ECD curves of a model molecule (**1a**). A butenolide derivative was originally misassigned to bear the same structure as that of **1**, but it was later revised. Thus, the compound **1** was reported as a new compound and was named 4-demethyl ester aspernolide N (**1**). The known compounds were identified as 3-hydroxy-5-[[4-hydroxy-3-(3-methyl-2-buten-1-yl)phenyl]methyl]-4-(4-hydroxyphenyl)-2(5H)-furanone (**2**), 4-(4-hydroxyphenyl)-5-(4-hydroxyphenylmethyl)-2-hydroxyfuran-2-one (**3**), versicolactone B (**4**), butyrolactone I (**5**), aspernolide A (**6**), butyrolactone IV (**7**), aspernolide O (**8**). Bioassay study suggested that compounds **2–6** had better inhibitory effects on α -glucosidase than that of the positive control acabose with IC₅₀ values ranging from 95 to 148 μ M.

Keywords: Butenolide derivatives; *Aspergillus terreus*; Inhibitory effects on α -glucosidase. © 2023 ACG Publications. All rights reserved.

1. Introduction

In recent years, fungal strains have been a source of chemically diverse and biologically active secondary metabolites. The *Aspergillus* strains are ubiquitous in the environment, which can be found in soil, decomposing plant matter, household dust, building materials, plants, food, and water. The genus comprises about 200 species, the common strains are *A. fumigatus*, *A. flavus*, *A. clavatus*, *A. parasiticus*, *A. oryzae*, *A. terreus*, *A. nidulans*, and *A. niger* [1]. *Aspergillus* strains could produce metabolites with complex structures or obvious biological activity, such as terpenoids [1-3], cytotoxic sterols [4], penolic C-glycosides [5], novel alkaloids [6-8], phenolic bisabolane sesquiterpenes [9], amide [10], cyclic peptides [11, 12], and indole glucoside [13].

The species *A. terreus* from marine resources was found to be prolific and, previous chemical investigations on the species led to the isolation of butyrolactones [14-16], meroterpenoids [17, 18], sesterterpenoids [19], alkaloids [20], and cyclic peptides [20]. Some of them exhibited outstanding bioactivities, particularly, the butenolide derivatives have been reported to possess noteworthy α -glucosidase inhibitory [21] and promising antiallergic effects [22].

In our research, we found that the ¹H NMR spectrum of the extract of a strain *Aspergillus terreus* exhibited the characteristic resonances of butyrolactones. Further purification of the extract afforded 8 butyrolactones. Compounds **1–8** were screened for inhibitory effects of α -glucosidase enzyme. Herein, we report the isolation, structure elucidation, and biological evaluation of the isolated compounds from the species.

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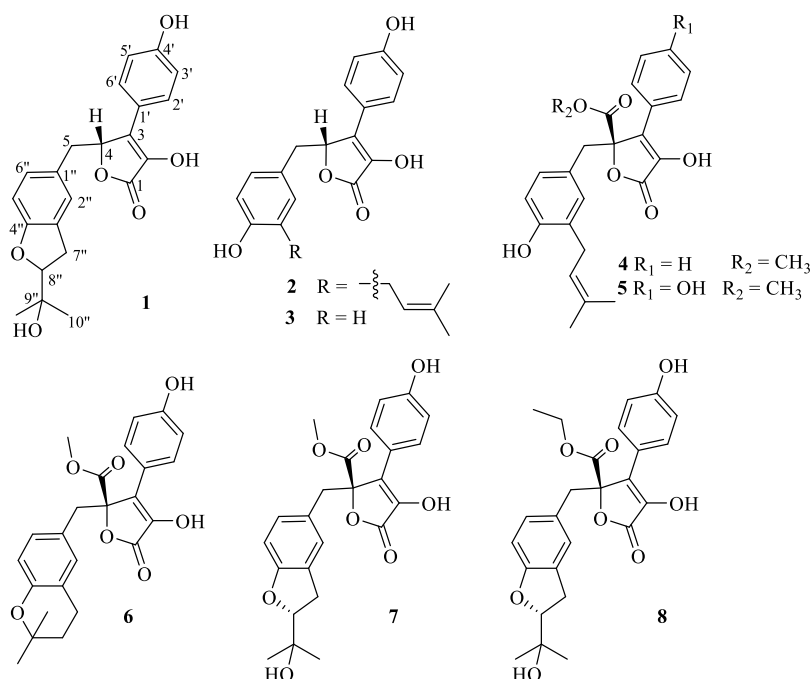
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Figure 1. Isolated compounds (1–8) from the marine-derived fungus *Aspergillus terreus* LPFH-SW1

2. Materials and Methods

2.1. General Experimental Procedures

UV spectrum was recorded on a Cary 300 spectrometer. The ¹H and ¹³C 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. Ethyl acetate (EtOAc, ACS grade), Methanol (MeOH, ACS Grade), Acetonitrile (ACN, ACS grade) and Ultrapure water used during the extraction process.

2.2. Microorganism Material

The fungal strain LPFH-SW1 was obtained from the sea sediments of Hangzhou Bay. It was identified to be *Aspergillus terreus* based on morphological features and by comparison of the ITS sequence region with that of a similar record in GenBank (MG575480.1). 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 40 Fernbach flasks (500 mL), each containing 90 g of rice. Distilled water (100 mL) was added to each flask, and the contents were soaked for 3 h and then were autoclaved at 15 psi for 30 min. Each flask was inoculated with 3.0 mL of the spore inoculum and incubated at room temperature for about a month. The fermented materials were extracted with ethyl acetate (EtOAc) (3 × 6000 mL) in an ultrasonic bath for 20 min. After evaporation under vacuum, the EtOAc extract (12.0 g) was subjected to an Octadecylsilyl (ODS) silica gel column chromatography eluted with MeOH/H₂O (20:80→100:0) to afford 10 fractions (F1–F10). F7 was separated into seven subsequent fractions (F7a–F7g) via C-18 silica gel column chromatography

(CC) that eluted with MeOH/H₂O (50:50→100:0). F7d was separated by HPLC using ACN/H₂O (71:29, 3 mL/min) to afford **4** (4.2 mg, *t_R* 20 min). F6 was further chromatographed over C-18 silica gel CC eluted with MeOH/H₂O (40:60→100:0) to afford 6 subfractions F6a–F6f. F6b was separated by HPLC using ACN/H₂O (52:48, 3 mL/min) to obtain **1** (7.2 mg, *t_R* 38 min). F6c was separated by HPLC using ACN/H₂O (56:44, 3 mL/min) to obtain **8** (109.5 mg, *t_R* 21 min) and **9** (34.7 mg, *t_R* 18 min). F6d was chromatographed by HPLC using ACN/H₂O (60:40, 3 mL/min) as eluent to obtain **2** (3.0 mg, *t_R* 26 min). F6e was separated by HPLC eluted with ACN/H₂O (60:40, 3 mL/min) to obtain **5** (2.3 mg, *t_R* 22 min) and **7** (2.5 mg, *t_R* 15 min). F5 was subjected to semi-preparative YMC-pack ODS-A column ACN/H₂O (55:45) to obtain **6** and subfractions (F5a–F5f). F5c was further purified by HPLC on a semi-preparative YMC-pack ODS-A column using MeOH/H₂O (65:35, 3 mL/min) to afford **3** (3.5 mg, *t_R* 31 min).

4-Demethyl ester aspernolide N (1): Yellowish oil; [α]_D²⁵ −32 (*c* 0.10, MeOH); UV (MeOH) λ_{\max} (log ϵ) 203 (4.52), 295 (4.21) nm; ECD (*c* 1.0 × 10^{−4} M, MeOH) λ_{\max} ($\Delta\epsilon$) 287 (−4.15), 231 (−5.62); ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 381.1327 [M−H][−] (calcd. for C₂₂H₂₁O₆[−], 381.1344).

2.4 α -Glucosidase Inhibitory Assay

The α -glucosidase (0.2 U) from *Saccharomyces cerevisiae* was diluted to 0.067 M phosphate buffer containing disodium hydrogen phosphate and potassium phosphate monobasic (pH 6.8). The test was performed in a 60 μ L reaction system consisting of α -glucosidase solution (20 μ L) and DMSO or sample (20 μ L). After incubation, PNPG (20 μ L, 4 mM) was added and was incubated for 15 min at 37 °C, and the reaction was quenched by the addition of Na₂CO₃ (60 μ L, 0.2 M). The absorbance was determined (405 nm). All assays were performed in three replicates, acarbose was used as the reference. Inhibition was determined according to the equation: Inhibition (%) = ((A_{Control} − A_{test})/A_{control}); IC₅₀ values are computed via concentration vs. percent inhibition values [13].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** had a molecular formula of C₂₂H₂₂O₆, as established by the HRESIMS and ¹³C NMR spectroscopy (Table 1), requiring 12 degrees of unsaturation. The ¹H NMR spectrum provided signals for two methyls [δ_{H} 1.21 (s) and 1.19 (s)], two oxygenated proton [δ_{H} 4.52 (dd, *J* = 9.2, 8.7 Hz, 1H); 5.57 (dd, *J* = 3.6, 5.3 Hz, 1H)], a 1,3,4-trisubstituted benzene ring [δ_{H} 6.76 (d, *J* = 1.6 Hz, 1H); 6.69 (d, *J* = 8.0, 1.6 Hz, 1H); 6.54 (d, *J* = 8.0 Hz, 1H)], a 1,4-disubstituted benzene ring [δ_{H} 7.58 (d, *J* = 8.7 Hz, 2H); 6.89 (d, *J* = 8.7 Hz, 2H)], and another four protons (δ_{H} 3.24, 3.06×2, 2.87) including two geminally coupled protons [δ_{H} 3.24 (1H, dd, *J* = 14.6, 3.6 Hz); 2.87 (1H, d, *J* = 14.6, 5.3 Hz)]. The ¹³C NMR and HSQC spectra exhibited 22 carbon resonances, including 15 aromatic carbons for two benzene rings, a double bond, and one carbonyl carbon, the remaining seven carbons were attributed to two methyls (δ_{C} 25.2, 25.3), two methylenes (δ_{C} 31.5, 39.8), two oxygen-bearing methines (δ_{C} 90.4, 80.3), and an oxygen-bearing tertiary carbon (δ_{C} 72.5). These structural features suggested a butenolide derivative, structurally similar to butyrolactone IV (**8**). A comparison of their NMR data revealed that the obvious differences between **1** and **8** were attributed to the absence of the methyl ester moiety and the presence of an additional oxygenated methine in **1**, suggesting that the methyl ester moiety attached to C-1 in **8** was replaced by a hydrogen atom in **1**.

The assumed structure of **1** was confirmed by detailed analyses of the 2D NMR data (Figure 2). Especially, the COSY relationship between the additional oxygenated methine proton at δ_{H} 5.57 (H-4) and the methylene protons H₂-5 (δ_{H} 3.24, 2.87) and the HMBC correlation from H-4 to C-1" certified the location of the methine CH-4 (δ_{H} 5.57; 80.3). The HMBC correlations from H-8" (δ_{H} 4.52) to C-3" (δ_{C} 128.0) and C-4" (δ_{C} 160.3) and H-7" (δ_{H} 3.06) to C-3" (δ_{C} 128.0), C-2" (δ_{C} 127.4), and C-4" (δ_{C} 160.3), and the COSY correlations between H-7" and H-8" (δ_{H} 4.52) indicated the

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presence of a furan ring consisting of C-3'', C-4'', C-7'' (δ_C 31.5), and C-8'' (δ_C 90.4). The presence of the furan ring could be confirmed by comparing the chemical shifts of C-2''–10'' with those of compounds 7–9.

Table 1. ^1H and ^{13}C NMR Data of **1** in Methanol- d_4 .^a

No.	1				
	δ_{H}	δ_{C}		δ_{H}	δ_{C}
1		171.8	1''		128.2
2		137.9	2''	6.76, d (1.6)	127.4
3		123.7	3''		128.0
4	5.57, dd (3.6, 5.3)	80.3	4''		160.3
5	3.24, dd (14.6, 3.6) 2.87, dd (14.6, 5.3)	39.8	5''	6.54, d (8.0)	109.2
1'		131.6	6''	6.69, d (8.0, 1.6)	130.4
2'	7.58, d (8.7)	130.4	7''	3.06, m	31.5
3'	6.89, d (8.7)	116.6	8''	4.52, dd (9.2, 8.7)	90.4
4'	6.89, d (8.7)	159.3	9''		72.5
5'		116.6	10''	1.19, s	25.3
6'	7.58, d (8.7)	130.4	11''	1.21, s	25.2

^a ^1H NMR recorded at 400 MHz, ^{13}C NMR recorded at 100 MHz.

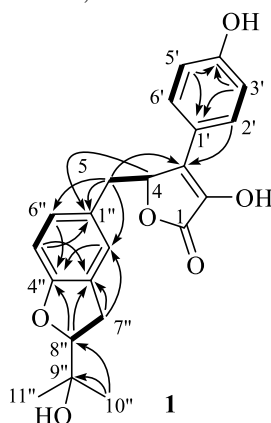


Figure 2. Key COSY (—) and HMBC (---) correlations of **1**

In order to assign the absolute configuration of C-4 (δ_C 80.3), the ECD calculation was performed at the b3lyp/6-31+g(d,p) level in methanol using the b3lyp/6-31+g(d,p)-optimized geometries for the model molecules (*R*-**1a** and *S*-**1a**). Comparison of the experimental CD curve of **1** with the computed ECD curves (Figure 3) indicated the absolute configurations of **1** to be 4*R*. The structure of **1** was thus determined as depicted. According to the literature [16, 23], a butenolide derivative was originally misassigned the same structure as **1** and was later revised. So compound **1** was a new compound and was named 4-demethyl ester aspernolide N

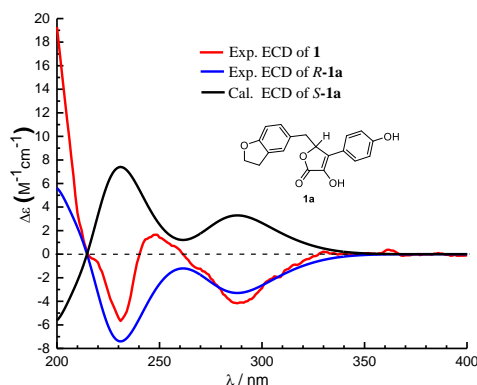


Figure 3. Experimental ECD spectrum of **1** in MeOH and the calculated ECD spectra of model molecules *R*-**1a** and *S*-**1a** at the b3lyp/6-31+g(d,p) level

Besides, compounds **2–8** were assigned to be 3-hydroxy-5-[[4-hydroxy-3-(3-methyl-2-buten-1-yl)phenyl]methyl]-4-(4-hydroxyphenyl)-2(5H)-furanone (**2**) [24], 4-(4-hydroxyphenyl)-5-(4-hydroxyphenylmethyl)-2-hydroxyfuran-2-one (**3**) [25], versicolactone B (**4**) [26], butyrolactone I (**5**) [27], aspernolide A (**6**) [27], butyrolactone IV (**7**) [28], aspernolide O (**8**) [28] by comparing the NMR data with those reported in the literature.

3.2. α -Glucosidase Inhibitory Effects of the Isolated Compounds

The literature revealed that butyrolactones had α -glucosidase inhibitory effect. The inhibitory effect of these compounds against α -glucosidase were tested at an initial concentration of 200 μ M following the procedures in the literature [13, 29]. Those exhibited inhibitions more than 50% were subsequently selected for further evaluation to calculate the IC_{50} values. The results showed that compounds **2–6** showed significant inhibitory effects with IC_{50} values less than 150 μ M (Table 2), being more active compared to the positive control acarbose (297 μ M). The structural similarity and the activity revealed some structure-activity relationship. It was found that, the isopentene group at C-3'' had no positive effect for the biological activity, since compound **3** was much more active than that of compound **2**. The hydroxyl group at C-4' seemed to contribute to the activity, since **4** was less active than its hydroxylated derivative **5**. The furan moiety (C-3'', C-4'', C-7'', C-8'') may led to a sharp decrease on the activity, since the activity of compounds **1, 7, 8** was much weaker than that of **2–6**.

Table 2. Inhibitory effects of the compounds **1–8** on α -glucosidase.

No.	Inhibition (%) ^a	IC_{50} (μ M)
1	17%	nt. ^b
2	56%	148
3	92%	95
4	71%	123
5	79%	115
6	65%	131
7	23%	nt. ^b
8	21%	nt. ^b
Acarbose		297

^a at 200 μ M, ^b not tested

Supporting Information

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