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BRIEF COMMUNICATION

Asperitaconic acids A–C, antibacterial itaconic acid derivatives produced by a marine-derived fungus of the genus *Aspergillus*

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Abstract

Three new itaconic acid derivatives, asperitaconic acids A–C (**1**–**3**), were isolated from the ethyl acetate extracts of the rice fermentation of a marine-derived fungus *Aspergillus niger*, and asperitaconic acids A–C were characterized by an itaconic acid unit and an alkyl chain moiety. Their structures were established by interpretation of their spectroscopic data including NMR and HRESIMS. Asperitaconic acids A–C exhibited antibacterial effect against *Staphylococcus aureus* with MIC values of 16–32 μg/mL, whereas these compounds showed no cytotoxicity against HepG2 and HeLa cancer cell lines.

Marine-derived *Aspergillus* genus, belonging to ascomycetes, has been regarded as a well-known producer of new bioactive natural products encompassing structurally diverse scaffolds with fascinating biological functions [1]. In recent years, species of the genus *Aspergillus* have been reported to produce a wide variety of bioactive metabolites such as polyketides [2], diketopiperazines [3], ergosteroids [4], xanthonoids [5], alkaloids [6], and lipopeptides [7]. In our continuous search for bioactive secondary metabolites from marine fungi, a large-scale culture of a marine-derived fungus, identified as *Aspergillus niger* LS11 in the rice medium led to the isolation of three new compounds, named asperitaconic acids A–C (**1**–**3**). In this paper, we presented the isolation, structure characterization, and biological activities of asperitaconic acids A–C.

The producing strain LS11 was isolated from a marine sponge *Haliclona* sp. collected from the Linshui, Hainan Province, China, and identified as *A. niger* based on the ITS sequencing. The fungal strain was first grown on potato dextrose agar plate (PDA, 8.0 g of potato extract, 20 g of glucose, 35 g of sea salt, and 20 g of agar in 1 L of distilled water) for 3 days. Agar plugs were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of PDB (8.0 g of potato extract, 20 g of glucose, and 35 g of sea salt in 1 L of distilled water) as seed culture at 28 °C in a shaker (150 rpm) for 3 days. A scaled-up fermentation was conducted on solid rice cultures in 1 L Erlenmeyer flasks (20 flasks; 160 mL of distilled water and 120 g of rice; autoclave). Each flask was inoculated with 10 mL of the seed culture and fermented at 28 °C without shaking for 30 days. The fungal culture was then extracted three times with EtOAc at room temperature. The resulting EtOAc extract was evaporated to dryness under reduced pressure to yield a crude extract (10.5 g). The extract was fractionated by ODS MPLC (30–100% methanol in water, flow rate 20 mL/min, 120 min) to obtain five subfractions (1−5). Fr. 3 was finally purified by reversed-phase HPLC using 40% MeCN/H_2O as eluent to afford compounds **1** (2 mg) and **2** (3 mg). Fr. 4 was separated by reversed-phase HPLC, eluting with MeCN/H_2O to give **3** (3 mg) (Fig. 1).

Asperitaconic acid A (**1**) was isolated as a colorless oil. Its molecular formula was deduced as $C_{12}H_{20}O_5$ based on the HRESIMS data at *m/z* 262.1648 $[M + NH_4]^+$, indicating 3° of unsaturation. The ^{13}C NMR in combination with the

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HSQC spectrum of **1** showed 12 carbon signals for two carbonyl groups at δ_C 173.8 (C-1) and 170.6 (C-4), two olefinic groups at δ_C 137.9 (C-3) and 128.9 (C-11), including one exo-methylene group, one methoxyl group at δ_C 52.1 (C-12), five methylene groups at δ_C 32.5 (C-9), 31.3 (C-5), 28.9 (C-7), 27.3 (C-6), and 25.4 (C-8), one oxymethylene group at δ_C 62.9 (C-10), along with one methine group at δ_C 46.2 (C-2) (Table 1). ^{1}H-NMR signals at δ_H 3.65 (*t*, $J = 7.6$ Hz, 2H), 1.57 (m, 2H), 1.34 (m, 2H) × 3, 1.91 (m, 1H), and 1.69 (m, 1H), each integrating for two protons indicated the existence of the 1-hexanol side chain. The detail NMR data suggested that **1** shared the same itaconic acid core structure as hexylitaconic acid, a known itaconic acid derivative obtained from the fungus *Aspergilus niger* [8], except that the presence of a hydroxyl group and a methoxyl group. The complete structure of **1** was subsequently accomplished by the ^{1}H–^{1}H COSY and HMBC spectra. The main difference was the hexyl side chain substituted by a hydroxyl group at C-10 in **1**, which was confirmed by the COSY cross-peaks of H_2-9/H_2-10 and H-5/H-6 and the HMBC correlations of H_2-9 with C-7 and C-8 and H_2-5 with C-7, respectively (Fig. 2). The location of the methoxyl group was further corroborated to be at C-12 by key HMBC cross-peak of H_3-12/C-1. The absolute configuration at C-2 was unambiguously assigned as *S* based on the value of the optical rotation $[\alpha]^{22}_D + 2.7$ (*c* 0.50, MeOH) for **1** and the *R*-(−)/*S*-(+) relationship [9]. Consequently, the structure of **1** was determined to be as shown in Fig. 1 and named asperitaconic acid A.

Asperitaconic acid B (**2**) was assigned to be a molecular formula of $C_{13}H_{20}O_6$ with 4° of unsaturation by the HRESIMS at *m/z* 290.1585 $[M + NH_4]^+$. A direct comparison of the ^{1}H and ^{13}C NMR data of **2** (Table 1) with those of **1** revealed that they share the similar structure, except that **2** has one more acetyl group at C-10 and one less methoxy group, which was further confirmed by the HMBC correlations of H_3-12 (δ_H 2.05, s)/C-11 (δ_C 171.8) and H_2-10 (δ_H 4.05, *t*, $J = 6.7$ Hz)/C-11 (Fig. 1). Finally, considering the optical rotation $[\alpha]^{22}_D + 7.70$ (*c* 2.50, MeOH), the absolute configuration at C-2 of **2** was established as *S*. Therefore, the structure of **2** was established as depicted in Fig. 1.

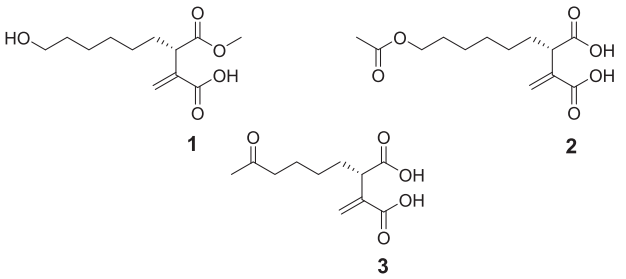

Fig. 1 Chemical structures of compounds **1**–**3**

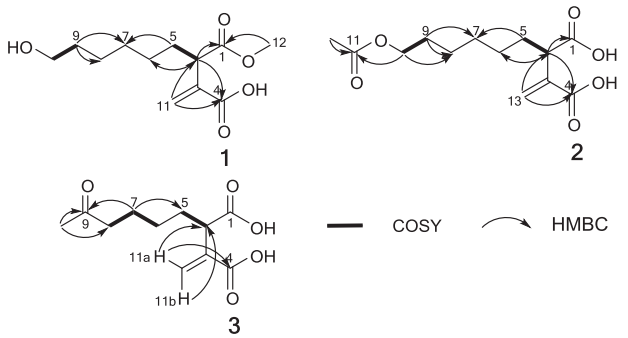

Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of asperitaconic acids A–C (**1**–**3**)

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data of **1**–**3** ($CDCl_3$)

No.	**1**		**2**		**3**	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	173.8, C		179.0, C		178.5, C	
2	46.2, CH	3.50 (t, 7.4)	46.7, CH	3.47 (t, 7.3)	47.2, CH	3.39 (m)
3	137.9, C		137.5, C		137.6, C	
4	170.6, C		171.3, C		171.0, C	
5	31.3, CH_2	1.91 (m); 1.69 (m)	30.3, CH_2	1.93 (m); 1.72 (m)	29.7, CH_2	1.93 (m); 1.73 (m)
6	27.3, CH_2	1.34 (m)	27.3, CH_2	1.35 (m)	26.8, CH_2	1.33 (m)
7	28.9, CH_2	1.34 (m)	29.0, CH_2	1.35 (m)	23.3, CH_2	1.60 (m)
8	25.4, CH_2	1.34 (m)	25.7, CH_2	1.35 (m)	43.3, CH_2	2.45 (d, 7.3)
9	32.5, CH_2	1.57 (m)	28.5, CH_2	1.61 (m)	209.5, C	
10	62.9, CH_2	3.65 (*t*, 7.6)	64.8, CH_2	4.05 (*t*, 6.7)	29.9, CH_3	2.14 (s)
11	128.9, CH_2	6.49 (s); 5.86 (s)	171.8, C		129.3, CH_2	6.48 (s); 5.81 (s)
12	52.1, CH_3	3.69 (s)	21.1, CH_3	2.05 (s)		
13			129.5, CH_2	6.53 (s); 5.87 (s)		

Asperitaconic acid C (**3**) was obtained as a colorless oil that presented a positive HRESIMS ion at *m/z* 246.1336 $[M + NH_4]^+$, corresponding to the molecular formula $C_{11}H_{16}O_5$ and 4° of unsaturation. The ^1H and ^{13}C NMR spectral data of **3** (Table 1) were closely related to those of **1**, indicating that **3** was a structural analog of **1**, with the exception of the absence of resonances for the hydroxyl and methoxy moiety and the appearance of resonance for the ketone group in **3**, which was supported by its molecular formula and resonance at δ_C 209.5 (C-9). This conclusion was further supported by the HMBC correlations from H_3-10 (δ_H 2.14) and H_2-7 (δ_H 1.60) to C-9. The optical rotation data $[\alpha]^{22}_D + 2.8$ (*c* 0.90, MeOH) observed for **3** defined the absolute configuration to be *S* as shown in Fig. 1 according to the *R*-(−)/*S*-(+) relationship [9].

Antimicrobial activities of compounds **1**−**3** were evaluated against clinically isolated *S. aureus* and *Escherichia coli* strains using broth microdilution in 96-well microplates [10]. Chloramphenicol was used as positive control. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test compound completely inhibiting visible growth of the test microorganisms. Compounds **1**−**3** had inhibitory effect on *S. aureus* with MIC values of 16, 32, and 32 μg/mL, respectively (chloramphenicol, MIC value of 4 μg/mL). However, compounds **1**−**3** were inactive against *E. coli* at the concentration of 64 μg/mL (chloramphenicol, MIC value of 2 μg/mL). Asperitaconic acids A–C (**1**−**3**) were assayed for cytotoxicity against HepG2 and HeLa cancer cell lines by the CCK-8 (Cell Counting Kit-8) method [11] using doxorubicin as positive control. However, compounds **1**−**3** were found to be noncytotoxic at the 100 μM concentration on both human cancer cell lines.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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