

Aspterrics A and B, New Sesquiterpenes from Deep Sea-derived Fungus *Aspergillus terreus* YPGA10

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Abstract: Two new sesquiterpenes, namely aspterric A (1) and aspterric B (2), together with aspterric acid (3), were isolated from the deep-sea-derived fungus *Aspergillus terreus* YPGA10. The structures of aspterrics A and B were determined by NMR and HRESIMS data. Biogenetically, aspterrics A and B were assumed to be the intermediates to derive aspterric acid.

Keywords: *Aspergillus terreus*; deep-sea-derived fungus; sesquiterpenoids; aspterrics A and B. © 2019 ACG Publications. All rights reserved.

1. Introduction

The fungus *Aspergillus terreus*, widely distributes in natural environment, has proved to be a potential fungus for producing molecules with significant bioactivity or complex structures. Previous studies of this fungus have led to the identification of meroterpenoids [1, 2], sesterterpenoids [3], butenolides [4-6], alkaloids [7], and cyclic peptides [8]. Some possess unique carbon skeletons and some exhibit remarkable bioactivities such as enzyme inhibition, antitumor activity, and antiviral activity. As part of our ongoing efforts to discover bioactive molecules from marine-derived fungi [9-11], chemical examination of a deep-sea sediment-derived *Aspergillus terreus* YPGA10 led to the isolation of a carotane-type sesquiterpene aspterric acid (3) and two new biogenetically related sesquiterpenes (1 and 2). Herein, the isolation and structural identification were described.

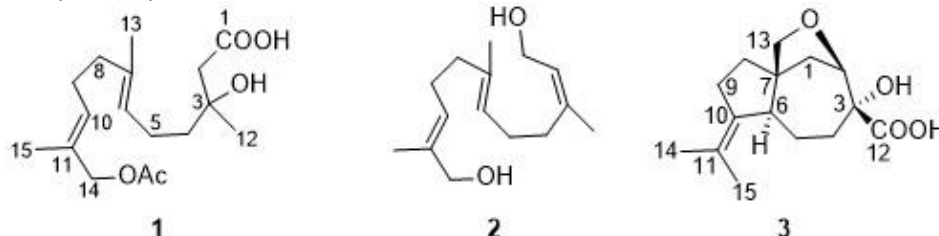


Figure 1. Structures of compounds 1–3 from *Aspergillus terreus* YPGA10

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2. Materials and Methods

2.1. Microorganism Material

Fungus *Aspergillus terreus* (YPGA10) was isolated from the deep-sea sediment collected in the Yap Trench at a depth of 4159 m. The strain was identified as *Aspergillus terreus* based on microscopic examination and by internal transcribed spacer (ITS) sequencing. The ITS sequence has been deposited in GenBank (<http://www.ncbi.nlm.nih.gov>) with accession number MG835907. The strain YPGA10 (MCCC 3A01013) was deposited at the Marine Culture Collection of China.

2.2. Fermentation and Isolation

The fermentation was conducted in 40 Fernbach flasks (500 mL), each containing 70 g of rice. Distilled H₂O (90 mL) was added and the contents were autoclaved at 15 psi for 30 min. After cooling to room temperature (r.t.), each flask was inoculated with 3.0 mL of the spore inoculum and incubated at r.t. for 40 days. The fermented material was extracted with EtOAc (4000 mL) for three times. After evaporation under vacuum, the EtOAc extract (12.0 g) was chromatographed by ODS silica gel column chromatography (CC) (MeOH/H₂O: 20:80→100:0) to give ten fractions (F1–F10). F7 (0.40 g) was subjected to HPLC on a semipreparative YMC-pack ODS-A column using MeOH/H₂O (65:35, 2 mL/min) to obtain seven fractions (F7a–F7g). F7f was purified by HPLC eluted with CH₃CN/H₂O (57:43, 2 mL/min) to obtain **1** (7.1 mg, *t_R* 35.5 min) and **2** (2.8 mg, *t_R* 39.5 min). F6 was subjected to Sephadex LH-20 CC using MeOH to give four fractions (F6a–F6d). F6a was separated on the YMC column with a mobile phase of CH₃CN/H₂O (53:47, 2 mL/min) to afford **3** (15 mg, *t_R* 27.5 min).

Aspterric A (**1**): Colorless oil; [α]_D²⁰ 0 (*c* 0.05, MeOH); ¹H NMR (methanol-*d*₄, 400 MHz) δ _H 2.45 (2H, s, H₂-2), 1.57 (2H, m, H₂-4), 2.09 (2H, m, H₂-5), 5.14 (1H, t, *J* = 7.1 Hz, H-6), 2.03 (2H, m, H₂-8), 2.16 (2H, m, H₂-9), 5.46 (1H, t, *J* = 7.1 Hz, H-10), 1.28 (3H, s, H₃-12), 1.63 (3H, s, H₃-13), 4.44 (2H, s, H₂-14), 1.65 (3H, s, H₃-15), 2.04 (3H, s, COCH₃). ¹³C NMR (methanol-*d*₄, 100 MHz) δ _C 175.9 (C-1), 46.5 (C-2), 72.1 (C-3), 42.7 (C-4), 23.5 (C-5), 125.9 (C-6), 135.6 (C-7), 40.1 (C-8), 27.1 (C-9), 130.3 (C-10), 131.4 (C-11), 27.2 (C-12), 16.0 (C-13), 71.3 (C-14), 14.0 (C-15), 172.9 (COCH₃), 20.8 (COCH₃). HRESIMS *m/z* 311.1853 [M – H][–] (calcd for C₁₇H₂₇O₅, 311.1864).

Aspterric B (**2**): Colorless oil; ¹H NMR (methanol-*d*₄, 400 MHz) δ _H 4.06 (2H, dd, *J* = 6.8, 0.8 Hz, H₂-1), 5.36 (1H, br t, *J* = 6.8 Hz, H-2), 2.10 (2H, m, H₂-4), 2.10 (2H, m, H₂-5), 5.15 (1H, t, *J* = 7.0 Hz, H-6), 2.02 (2H, m, H₂-8), 2.14 (2H, m, H₂-9), 5.39 (1H, t, *J* = 7.0 Hz, H-10), 1.75 (3H, s, H₃-12), 1.63 (3H, s, H₃-13), 3.91 (2H, s, H₂-14), 1.65 (3H, s, H₃-15). ¹³C NMR (methanol-*d*₄, 100 MHz) δ _C 59.2 (C-1), 125.7 (C-2), 139.6 (C-3), 32.9 (C-4), 27.6 (C-5), 125.2 (C-6), 136.3 (C-7), 40.5 (C-8), 27.3 (C-9), 126.5 (C-10), 135.9 (C-11), 23.7 (C-12), 16.1 (C-13), 68.9 (C-14), 13.7 (C-15). HRESIMS *m/z* [M + Na]⁺ 261.1826 (calcd for C₁₅H₂₆O₂Na⁺, 261.1825).

Aspterric acid (**3**): Colorless oil; ¹³C NMR (methanol-*d*₄, 100 MHz) δ _C 35.2 (C-1), 84.8 (C-2), 76.4 (C-3), 33.1 (C-4), 36.7 (C-5), 56.7 (C-6), 54.2 (C-7), 24.8 (C-8), 35.0 (C-9), 136.7 (C-10), 125.1 (C-11), 177.7 (C-12), 79.4 (C-13), 21.0 (C-14), 23.4 (C-15).

3. Results and Discussion

The molecular formula of compound **1** was determined to be C₁₇H₂₈O₅ by the HRESIMS data, requiring four degrees of unsaturation. The ¹H NMR and HSQC spectra displayed signals for four methyl singlets [δ _H 1.28 (3H, s, H₃-12), 1.65 (3H, s, H₃-13), 1.67 (3H, s, H₃-15), 2.04 (3H, s, COCH₃)], two olefinic protons [δ _H 5.14 (1H, t, *J* = 7.1 Hz, H-6), 5.46 (1H, t, *J* = 7.1 Hz, H-10)], and six methylenes [δ _H 2.45 (2H, s, H₂-2), 1.57 (2H, m, H₂-4), 2.09 (2H, m, H₂-5), 2.03 (2H, m, H₂-8), 2.16 (2H, m, H₂-9), 4.44 (2H, s, H₂-14)]. The ¹³C NMR spectrum exhibited a total of 17 carbon resonances, including four olefinic carbons [δ _C 125.9, 135.6, 130.3, 131.4] for two double bonds, a

carbonyl carbon [δ_C 172.9 (COCH₃)] for an acetyl carbonyl carbon, and a carboxylic acid group [δ_C 175.9 (C-1)]. The two carbonyl carbons and two double bonds accounted for all four degrees of unsaturation, indicating **1** to be acyclic. The structure was further established to be a new farnesol derivative by 2D NMR data (Figure 2). The HMBC correlations from H₃-12 (δ_H 1.28) to C-2 (δ_C 46.5), C-3 (δ_C 72.1), C-4 (δ_C 42.7) and H₂-2 (δ_H 2.45) to the carboxylic acid carbon (δ_C 175.9) established a 3-hydroxy-3-methylbutanoic acid moiety (unit A). The COSY relationship from H₂-5 (δ_H 2.09) to H-6 (δ_H 5.14) and HMBC correlations from H₃-13 (δ_H 1.63) to C-6 (δ_C 125.9), C-7 (δ_C 135.6), and C-8 (δ_C 40.1) established an isopentenyl unit (unit B). Additional HMBC correlations from H₂-14 (δ_H 4.44) to C-10 (δ_C 130.3), C-11 (δ_C 131.4), C-15 (δ_C 14.0) and the COSY relationship between H₂-9 (δ_H 2.16) and H-10 (δ_H 5.46) established another isopentenyl unit (Unit C). The above three units were connected by the COSY correlations from H₂-8 to H₂-9 and from H₂-4 to H₂-5. The remaining acetyl group was located at C-14 (δ_C 71.3) by the HMBC correlation from H₂-14 to the acetyl carbonyl carbon (δ_C 172.9). The strong NOESY correlations of H₃-13/H₂-5, H₂-8/H-6, H₃-15/H₂-9, and H₂-14/H-10 indicated that both Δ^6 and Δ^{10} had an *E* configuration. The specific rotation of **1** ($[\alpha]_D^{20}$ 0) indicated that **1** was racemic. Compound **1** was given the trivial name aspterric A.

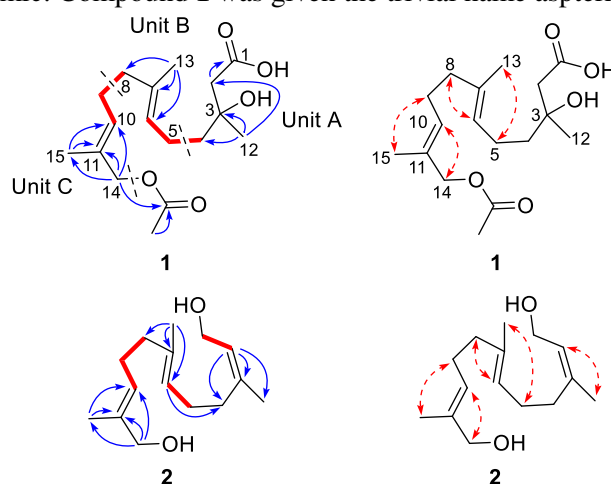


Figure 2. ¹H-¹H COSY (—), HMBC (—), and NOE (---) correlations of **1** and **2**.

Compound **2** had the molecular formula C₁₅H₂₆O₂ based on the HRESIMS and NMR data, indicating three degrees of unsaturation. The ¹H NMR and HSQC spectra displayed the signals for three olefinic protons [δ_H 5.36 (1H, t, *J* = 6.8 Hz, H-2), 5.15 (1H, t, *J* = 7.0 Hz, H-6), 5.39 (1H, t, *J* = 7.0 Hz, H-10)], three olefinic methyls [δ_H 1.63 (3H, s, H₃-13), 1.65 (3H, s, H₃-15), 1.75 (3H, s, H₃-12)], and six methylenes [δ_H 4.06 (2H, dd, *J* = 6.8, 0.8 Hz, H₂-1), 2.10 (2H, m, H₂-4), 2.10 (2H, m, H₂-5), 2.02 (2H, m, H₂-8), 2.14 (2H, m, H₂-9), 3.91 (2H, s, H₂-14)]. The ¹³C NMR spectrum, in combination with HSQC spectrum, resolved 15 carbon resonances attributable to three double bonds, three methyls, and six methylenes. The aforementioned data were similar to those of **1**, indicating a farnesol derivative [12]. The structure was further established by detailed 2D NMR analyses (Figure 2). The HMBC and COSY correlations established the same units B and C as those of **1**, while unit A was determined to be an isopentenyl alcohol moiety by the HMBC correlations from the additional olefinic proton H-2 (δ_H 5.36) to C-3 (δ_C 139.6), C-4 (δ_C 32.9), and C-12 (δ_C 23.7) and between its COSY relationship with the extra oxygenated methylene protons H₂-1 (δ_H 4.08). The three moieties were connected by the HMBC correlation from H-6 (δ_H 5.15) to C-4 (δ_C 32.9) and COSY relationship from H₂-8 (δ_H 2.02) to H-9 (δ_H 2.14). The strong NOESY correlations of H₃-13 (δ_H 1.63)/H₂-5 (δ_H 2.10), H₂-8/H-6, H₃-15 (δ_H 1.65)/H₂-9 (δ_H 2.14), H₂-14 (δ_H 3.91)/H-10 (δ_H 5.39), H₂-1 (δ_H 4.08)/H₂-4 (δ_H 2.10), H-2 (δ_H 5.36)/H₃-12 (δ_H 1.75) indicated that both Δ^6 and Δ^{10} had an *E* configuration, while Δ^2 had a *Z* configuration. Compound **2** was named aspterric B.

Compound **3** was identified to be aspterric acid by careful comparison with NMR data and specific rotation reported in the literature [13].

4. Conclusion

Three sesquiterpenoids including two new farnesol derivatives were isolated from the deep-sea-derived fungus *Aspergillus terreus* YPGA10. The structures of aspterrics A and B were established by detailed analyses of the NMR data and HRESIMS data. Biogenetically, aspterrics A and B were assumed to be the intermediates to derive aspterric acid.

Acknowledgments

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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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