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

Yuanli Li [1], Wan Liu [1], Wei Xu [2], Xi Zeng [1], Zhongbin Cheng [1,3*] and Qin Li [1,3*]

[1]*Pharmaceutical College, Henan University, Kaifeng 475004, People's Republic of China*

[2]*Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, People's Republic of China*

[3]*Eucommia ulmoides Cultivation and Utilization of Henan Engineering Laboratory, Kaifeng 475004, People's Republic of China*

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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.

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.

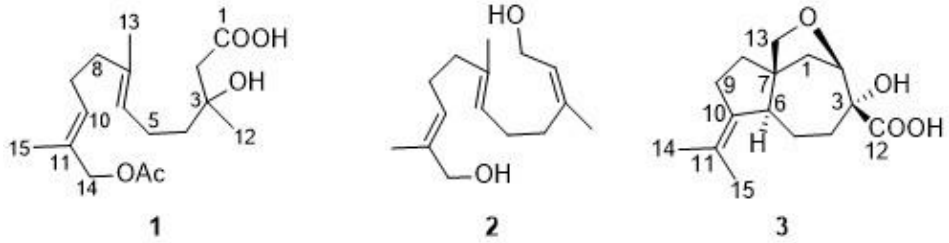

Figure 1. Structures of compounds **1**–**3** from *Aspergillus terreus* YPGA10

* Corresponding author: E-Mail: czb360@126.com; liqin6006@163.com.

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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 H_2O (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/H_2O: 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/H_2O (65:35, 2 mL/min) to obtain seven fractions (F7a–F7g). F7f was purified by HPLC eluted with CH_3CN/H_2O (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 CH_3CN/H_2O (53:47, 2 mL/min) to afford **3** (15 mg, t_R 27.5 min).

Aspterric A (**1**): Colorless oil; $[\alpha]^{20}_D$ 0 (*c* 0.05, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) δ_H 2.45 (2H, s, H_2-2), 1.57 (2H, m, H_2-4), 2.09 (2H, m, H_2-5), 5.14 (1H, t, *J* = 7.1 Hz, H-6), 2.03 (2H, m, H_2-8), 2.16 (2H, m, H_2-9), 5.46 (1H, t, *J* = 7.1 Hz, H-10), 1.28 (3H, s, H_3-12), 1.63 (3H ,s, H_3-13), 4.44 (2H, s, H_2-14), 1.65 (3H, s, H_3-15), 2.04 (3H, s, CO$\underline{CH_3}$). ^{13}C 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 ($\underline{CO}CH_3$), 20.8 (CO$\underline{CH_3}$). HRESIMS *m*/*z* 311.1853 $[M - H]^-$ (calcd for $C_{17}H_{27}O_5$, 311.1864).

Aspterric B (**2**): Colorless oil; ^{1}H NMR (methanol-d_4, 400 MHz) δ_H 4.06 (2H, dd, *J* = 6.8, 0.8 Hz, H_2-1), 5.36 (1H, br t , *J* = 6.8 Hz, H-2), 2.10 (2H, m, H_2-4), 2.10 (2H, m, H_2-5), 5.15 (1H, t, *J* = 7.0 Hz, H-6), 2.02 (2H, m, H_2-8), 2.14 (2H, m, H_2-9), 5.39 (1H, t, *J* = 7.0 Hz, H-10), 1.75 (3H, s, H_3-12), 1.63 (3H ,s, H_3-13), 3.91 (2H, s, H_2-14), 1.65 (3H, s, H_3-15). ^{13}C 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 $C_{15}H_{26}O_2Na^+$, 261.1825).

Aspterric acid (**3**)*:* Colorless oil; ^{13}C 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 $C_{17}H_{28}O_5$ by the HRESIMS data, requiring four degrees of unsaturation. The ^{1}H NMR and HSQC spectra displayed signals for four methyl singlets [δ_H 1.28 (3H, s, H_3-12), 1.65 (3H ,s, H_3-13), 1.67 (3H, s, H_3-15), 2.04 (3H, s, CO$\underline{CH_3}$)], 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, H_2-2), 1.57 (2H, m, H_2-4), 2.09 (2H, m, H_2-5), 2.03 (2H, m, H_2-8), 2.16 (2H, m, H_2-9), 4.44 (2H, s, H_2-14)]. The ^{13}C 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 (COCH$_3$)] 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 H$_3$-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 H$_2$-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 H$_2$-5 (δ_H 2.09) to H-6 (δ_H 5.14) and HMBC correlations from H$_3$-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 H$_2$-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 H$_2$-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 H$_2$-8 to H$_2$-9 and from H$_2$-4 to H$_2$-5. The remaining acetyl group was located at C-14 (δ_C 71.3) by the HMBC correlation from H$_2$-14 to the acetyl carbonyl carbon (δ_C 172.9). The strong NOESY correlations of H$_3$-13/H$_2$-5, H$_2$-8/H-6, H$_3$-15/H$_2$-9, and H$_2$-14/H-10 indicated that both Δ^6 and Δ^{10} had an *E* configuration. The specific rotation of **1** ([α]$^{20}_D$ 0) indicated that **1** was racemic. Compound **1** was given the trivial name aspterric A.

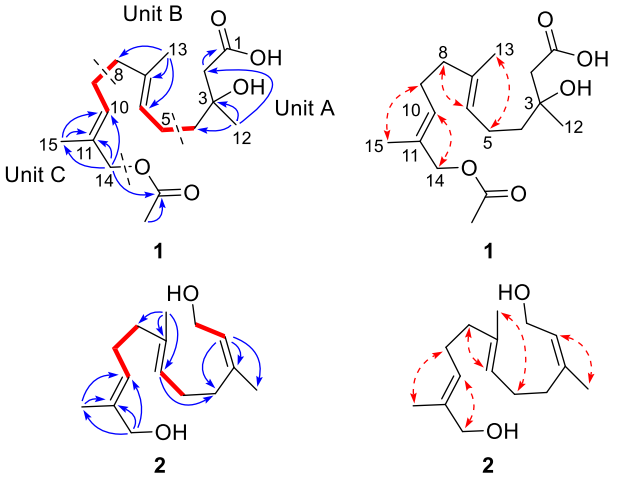

Figure 2. ^{1}H-^{1}H COSY (—), HMBC (⤳), and NOE (⇢) correlations of **1** and **2**.

Compound **2** had the molecular formula C$_{15}$H$_{26}$O$_2$ based on the HRESIMS and NMR data, indicating three degrees of unsaturation. The ^{1}H NMR and HSQC spectra displayed the signals for three 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, H$_3$-13), 1.65 (3H, s, H$_3$-15), 1.75 (3H, s, H$_3$-12)], and six methylenes [δ_H 4.06 (2H, dd, *J* = 6.8, 0.8 Hz, H$_2$-1), 2.10 (2H, m, H$_2$-4), 2.10 (2H, m, H$_2$-5), 2.02 (2H, m, H$_2$-8), 2.14 (2H, m, H$_2$-9), 3.91 (2H, s, H$_2$-14)]. The ^{13}C 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 H$_2$-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 H$_2$-8 (δ_H 2.02) to H-9 (δ_H 2.14). The strong NOESY correlations of H$_3$-13 (δ_H 1.63)/H$_2$-5 (δ_H 2.10), H$_2$-8/H-6, H$_3$-15 (δ_H 1.65)/H$_2$-9 (δ_H 2.14), H$_2$-14 (δ_H 3.91)/H-10 (δ_H 5.39), H$_2$-1 (δ_H 4.08)/H$_2$-4 (δ_H 2.10), H-2 (δ_H 5.36)/H$_3$-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**.**

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Supporting Information

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

ORCID
Yuanli Li:0000-0001-6953-720X
Wan Liu:0000-0001-5612-9434
Wei Xu:0000-0002-3265-7475
Xi Zeng:0000-0001-8742-1390
Zhongbin Cheng: 0000-0003-0942-6422
Qin Li:0000-0001-8295-6230

References

[1] C. Qi, M. Liu, Q. Zhou, W. Gao, C. Chen, Y. Lai, Z. Hu, Y. Xue, J. Zhang, D. Li, X.-N. Li, Q. Zhang, J. Wang, H. Zhu and Y. Zhang (2018). BACE1 inhibitory meroterpenoids from *Aspergillus terreus*, *J Nat. Prod*. **81**, 1937-1945.

[2] C.-C. Liaw, Y.-L. Yang, C.-K. Lin, J.-C. Lee, W.-Y. Liao, C.-N. Shen, J.-H. Sheu and S.-H. Wu (2015). New meroterpenoids from *Aspergillus terreus* with inhibition of cyclooxygenase-2 expression, *Org. Lett.* **17**, 2330-2333.

[3] Z. Liu, Y. Chen, S. Chen, Y. Liu, Y. Lu, D. Chen, Y. Lin, X. Huang and Z. She (2016). Aspterpenacids A and B, two sesterterpenoids from a mangrove endophytic fungus *Aspergillus terreus* H010, *Org. Lett.* **18**, 1406-1409.

[4] Y. Sun, J. Liu, L. Li, C. Gong, S. Wang, F. Yang, H. Hua and H. Lin (2018). New butenolide derivatives from the marine sponge-derived fungus *Aspergillus terreus*, *Bioorg. Med. Chem. Lett*. **28**, 315-318.

[5] K. Sun, G. Zhu, J. Hao, Y. Wang and W. Zhu (2018). Chemical-epigenetic method to enhance the chemodiversity of the marine algicolous fungus, *Aspergillus terreus* OUCMDZ-2739, *Tetrahedron* **74**, 83-87.

[6] C. Qi, W. Gao, D. Guan, J. Wang, M. Liu, C. Chen, H. Zhu, Y. Zhou, Y. Lai, Z. Hu, Q. Zhou and Y. Zhang (2018). Butenolides from a marine-derived fungus *Aspergillus terreus* with antitumor activities against pancreatic ductal adenocarcinoma cells, *Bioorg. Med. Chem*. **26**, 5903-5910.

[7] S. Cai, L. Du, A.L. Gerea, J.B. King, J. You, R.H. Cichewicz (2013). Spiro fused diterpene-indole alkaloids from a creek-bottom-derived *Aspergillus terreus*, *Org. Lett.* 15, 4186-4189.

[8] F. He, J. Bao, X. Y. Zhang, Z. C. Tu, Y. M. Shi and S. H. Qi (2013). Asperterrestide A, a cytotoxic cyclic tetrapeptide from the marine-derived fungus *Aspergillus terreus* SCSGAF0162, *J Nat. Prod.* **76**, 1182-1186.

[9] L. Liu, W. Xu, S. Li, M. Chen, Y. Cheng, W. Yuan, Z. Cheng and Q. Li (2018). Penicindopene A, a new indole diterpene from the deep-sea fungus *Penicillium* sp. YPCMAC1, *Nat. Prod. Res*. Doi:10.1080/14786419.2018.1514402.

[10] Z. Cheng, W. Xu, L. Liu, S. Li, W. Yuan, Z. Luo, J. Zhang, Y. Cheng and Q. Li (2018). Peniginsengins B–E, new farnesylcyclohexenones from the deep sea-derived fungus *Penicillium* sp. YPGA11, *Mar. Drugs* **16**, E358.

[11] Z. Cheng, W. Xu, Y. Wang, S. Bai, L. Liu, Z. Luo, W. Yuan and Q. Li (2019). Two new meroterpenoids and two new monoterpenoids from the deep sea-derived fungus *Penicillium* sp. YPGA11, *Fitoterapia* **133,** 120-124.

[12] P. Lai, H. Rao and Y. Gao (2018). Chemical composition, cytotoxic, antimicrobial and antioxidant activities of essential oil from *Anthriscus caucalis* M. Bieb grown in China, *Rec. Nat. Prod*. **12**, 290-294.

[13] A. Shimada, M. Kusano, S. Takeuchi, S. Fujioka, T. Inokuchi and Y. Kimura (2002). Aspterric acid and 6-hydroxymellein, inhibitors of pollen development in *Arabidopsis thaliana*, produced by *Aspergillus terreus*, *Z. Naturforsch. C* **57**, 459-464.