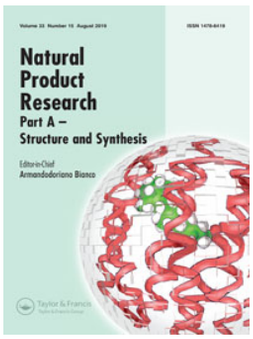

Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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To cite this article: Pei-Liang Zhang, Gang Wang, Feng-Qing Xu, Jin-Song Liu, Ju-Tao Wang, Rui Zhang, Hai-Tao Liu, Jiang-Miao Hu, Guo-Kai Wang & Pei-Yun Wu (2019) Aspergilolide, a steroid lactone produced by an endophytic fungus *Aspergillus sp*. MBL1612 isolated from *Paeonia ostii*, Natural Product Research, 33:15, 2133-2138, DOI: 10.1080/14786419.2018.1488706

To link to this article: https://doi.org/10.1080/14786419.2018.1488706

NATURAL PRODUCT RESEARCH
2019, VOL. 33, NO. 15, 2133–2138
https://doi.org/10.1080/14786419.2018.1488706

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Aspergilolide, a steroid lactone produced by an endophytic fungus *Aspergillus sp*. MBL1612 isolated from *Paeonia ostii*

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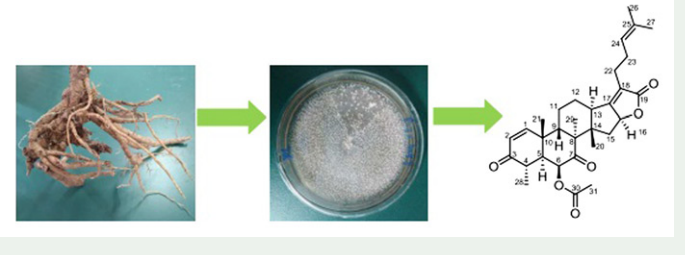
ABSTRACT

A new steroid lactone aspergilolide (**1**), and nine known compounds helvolic acid (**2**), verruculogen (**3**), tryprostatin B (**4**), 13-oxofumitremorgin B (**5**), fumitremorgin C (**6**), demethoxy fumitremorgin C (**7**), terezine D (**8**), aszonalenin (**9**), 12, 13-dihydroxy-fumitremorgin C (**10**) from cultures of the endophytic fungus *Aspergillus sp*. MBL1612. Their chemical structures were determined by a series of extensive spectroscopic methods. All of the compounds were isolated from this genus for the first time. The cytotoxicity against five human cancer cell lines of new compound were detected.



ARTICLE HISTORY
Received 15 April 2018
Accepted 10 June 2018

KEYWORDS
Edophytic fungus; *Aspergillus sp*. MBL1612; aspergilolide; cytotoxicity

1. Introduction

Recently, the studies have shown that the presence of many active substances in the metabolites of endophytic fungus is universal (Khan et al. 2015; Liu et al. 2015, 2017; El-Amrani et al. 2016; Zhu et al. 2016). And there are also many types of secondary metabolites in endophytic fungus including terpenes, quinones, alkaloids, steroids and polypeptides, and these compounds display various biological activities such as

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1488706.

antimicrobial, antitumor, antiviral (Shao et al. 2010; Brock et al. 2013; Chen et al. 2017; Cho et al. 2017; Yu et al. 2017). In continuation of our search for intriguingly structured bioactive molecules from endophytic fungus (Yu et al. 2017; Zhang et al. 2018), we performed chemical investigation on the fungal strain *A.* sp. MBL1612, of which secondary metabolites have not been researched until that was first reported in 2017 (Bashir et al. 2017). As a result, a new steroid lactone, namely, aspergilolide (**1**), as well as nine known, was isolated and identified from the culture extract of the fungal strain. These known compounds were identified as helvolic acid (**2**) (Sun et al. 2014; Luo et al. 2017), verruculogen (**3**) (Yang et al. 2013), tryprostatin B (**4**) (Zhao et al. 2006), 13-oxofumitremorgin B (**5**) (Luo et al. 2017), fumitremorgin C (**6**) (Ren et al. 2011), demethoxy fumitremorgin C (**7**) (Zhao et al. 2006), terezine D (**8**) (Wang et al. 1995), aszonalenin (**9**) (Yin et al. 2009), 12,13-dihydroxy-fumitremorgin C (**10**) (Luo et al. 2017).

2. Results and discussion

Compound **1** was obtained as white powder, has molecular formula $C_{31}H_{40}O_6$ as determined on the HR-ESI-MS (*m/z* 543.2518, $[M + Cl]^-$, calcd for 543.2519), indicating twelve degrees of unsaturation. The IR absorptions at 1750 cm^{-1} indicated the presence of carbonyl (C=O) groups. The UV spectrum showed maximum absorption at 208, 225 nm. The 1D NMR signals (Table S1) were attributed to seven methyl ($\delta_{C/H}$ 20.4/0.82, 27.7/1.42, 25.8/1.68, 18.0/1.60, 13.3/1.29, 18.4/1.31 and 20.9/2.11), five methylenes (δ_C 23.9, 22.1, 36.5, 24.3, 27.4), nine methines ($\delta_{C/H}$ 156.7/7.31, 128.1/5.88, 40.5/2.75, 47.2/2.31, 73.3/5.28, 42.1/2.57, 43.5/2.95, 81.6/4.93 and 123.2/5.11) including two oxygenated methines ($\delta_{C/H}$ 73.3/5.28, 81.6/4.93) and three olefinic methines ($\delta_{C/H}$ 156.7/7.31, 128.1/5.88 and 123.2/5.11), and ten quaternary carbon atoms (δ_C 201.2, 208.5, 52.4, 38.4, 53.1, 167.3, 125.2, 176.1, 133.2, 168.9) including four carbonyl carbon (δ_C 201.2, 208.5, 176.1, 168.9). Those data indicated that the skeleton of **1** was similar to *helvolic acid* (compound **2**). In comparison with **2**, the main differences is that the acetate group at position C-16 and the carboxyl group at position C-20 in **2** were condensed as an α, β-unsaturated γ-lactone ketone in **1**, which were further confirmed and established the planar structure of **1** by 2D NMR. According to the ^1H–^1H COSY spectrum, the following cross-peak H-1 (δ_H 7.31)/H-2 (δ_H 5.88), H-5 (δ_H 2.31)/H-4 (δ_H 2.75)/H-28 (δ_H 1.29), H-9 (δ_H 2.57)/H-11 (δ_H 2.23)/H-12 (δ_H 2.01, 1.81)/H-13 (δ_H 2.95), H-15 (δ_H 2.48, 1.76)/H-16 (δ_H 4.93) and H-23 (δ_H 2.23)/H-24 (δ_H 5.11) were displayed, for another, in the HMBC spectrum, some key long-range correlations were assigned by the HMBC correlations from H-21 (δ_H 1.42) to C-1 (δ_C 156.7), C-9 (δ_C 42.1), H-6 (δ_H 5.28) and H-31 (δ_H 2.11) to C-30 (δ_C 168.9), H-29 (δ_H 1.31) to C-7 (δ_C 208.5), H-20 (δ_H 0.82) to C-8 (δ_C 52.4), H-26 (δ_H 1.68) and H-27 (δ_H 1.60) to C-24 (δ_C 123.2), H-26 (δ_H 1.68) to C-27 (δ_C 18.0), H-13 (δ_H 2.95) to C-18 (δ_C 125.2) and H-22 (δ_H 2.38) to C-17 (δ_C 167.3), C-19 (δ_C 176.1). In addition, the HMBC correlations from H-1 (δ_H 7.31), H-4 (δ_H 2.75) to C-3 (δ_C 201.2) and H-1 (δ_H 7.31) to C-5 (δ_C 47.2), along with the cross-peak H-1 (δ_H 7.31)/H-2 (δ_H 5.88) and H-5 (δ_H 2.31)/H-4 (δ_H 2.75)/H-28 (δ_H 1.29) exhibited the part of ring A; The correlations from H-6 (δ_H 5.28) to C-5 (δ_C 47.2), C-7 (δ_C 208.5), C-10 (δ_C 38.4) and H-9 (δ_H 2.57) to C-7 (δ_C 208.5), C-10 (δ_C 38.4) built the part of ring B; The

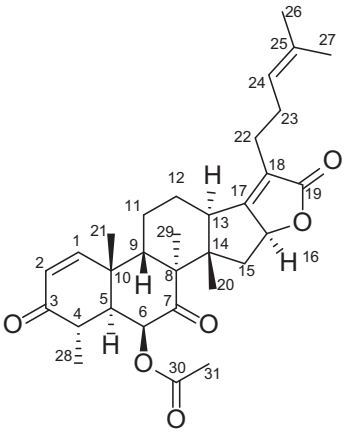

Figure 1. Chemical structure of compound **1**.

correlations from H-9 (δ_H 2.57) to C-14 (δ_C 53.1) and H-13 (δ_H 2.95) to C-8 (δ_C 52.4) along with the cross-peak H-9 (δ_H 2.57)/H-11 (δ_H 2.23)/H-12 (δ_H 2.01, 1.81)/H-13 (δ_H 2.95) displayed the part of ring C; The correlations from H-13 and H-16 to C-17 and H-20 to C-15 along with the cross-peak H-15 (δ_H 2.48, 1.76)/H-16 (δ_H 4.93) showed the part of ring D; As all the above functionalities accounted for eleven of the twelve degrees of unsaturation, with remained one degree of unsaturation taken into account along with the HMBC correlations from H-13 (δ_H 2.95) to C-18 (δ_C 125.2) and H-22 (δ_H 2.38) to C-17 (δ_C 167.3) and C-19 (δ_C 176.1) suggested that there is an α, β-unsaturated γ-lactone ketone on the side of ring D. Thus, the planar structure of compound **1** was established by foregoing evidence.

The relative configuration was determined by ROESY spectrum. In the ROESY spectrum of **1**, the correlations from H-4/H-21/H-9/H-20 and H-6/H-28/H-13/H-29 and H-5/H-29/H-15a/H-16 showed the same configuration with the *helvolic acid* (compound **2**) (Tschen et al. 1997; Sun et al. 2014; Luo et al. 2017). Finally, the structure of **1** was established as (4*R*, 5*R*, 6*S*, 8*R*, 9*S*, 13*R*, 14*S*)-20, 21, 28, 29-tetramethyl-3, 7, 19-trioxo-1, 2, 4, 5, 6, 9, 11, 12, 13, 15-tetradecahydro-1H-indeno[19, 18-b]furan-6-yl acetate (Figure 1), and named aspergilolide.

The new compound was evaluated in vitro for the cytotoxicity against five cancer cell lines including human lung adenocarcinoma A549 cell, breast cancer MDA-MB-231 and MCF-7 cells as well as nasopharyngeal carcinoma KB and KB-VIN cell lines. Unfortunately, the IC_{50} values (>40 μM) showed the new compound has no significant activity for five human cancer cell lines.

3. Experimental

3.1. General experimental procedures

Optical rotation was measured on SEPA 300 polarimeter (Horiba, Japan). UV spectra were recorded on Shimadzu 2401A spectrophotometer (Shimadzu, Japan) equipped with a DAD and a 1 cm path-length cell. IR spectra were obtained with BRUKER TENSOR 27 FT-IR (Bruker Corporation, Germany) with KBr pellets. The ^{1}H and ^{13}C NMR

were recorded by Bruker DRX-500 and AM-400 spectrometer (Karlsruhe, Germany), using tetramethylsilane as an internal standard. Chemical shifts were expressed in ppm with reference to the solvent signals. HR-ESI-MS spectra were measured on Shimadzu IT-TOF spectrometer. HPLC analyses were performed on an Agilent 1100 series and the ODS column used was a 150 × 4.9 mm i.d., 5 μm, Agilent Zorbax SB C18 column (America). Preparative HPLC were performed on an Agilent 1260 with the ODS column was an Agilent Zorbax SB C18 column (5 μm, 9.4 × 150 mm, America). MPLC was performed on a BÜCHI Sepacore system, the column was packed with Chromatorex C-18 (40–75 mm, Fuji Silysia Chemical Ltd., Japan). Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Sweden). Silica gel GF254 (Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) was used to monitor fractions from column chromatography.

3.2. Fungal strain

The endophytic fungus *Aspergillus sp*. MBL1612 was isolated from the fresh root bark of *Paeonia ostii* collected in March 2016 in Anhui Province, China by Dr. C.W. Fang, and a voucher specimen (No. F04013) was deposited in Anhui University of Chinese Medicine. The fungus was identified by analysis of its ITS region of the rDNA. (Kjer et al. 2010). After amplification and sequencing of the rDNA, the sequence data was submitted to GenBank, and a BLAST search result showed the most similar (99%) to the sequence of *Aspergillus sp*. MBL1612 (Compared to accession NO. KM924435.1).

3.3. Fermentation, extraction and isolation

The fungal strain was cultured on PDA and maintaineuntil discrete fungal cod at 28 °C lonies appeared. Then, pieces of fresh mycelium were inoculated in liquid PDB medium. Fermentation was carried out in 1000 mL flasks each containing 500 mL of medium on a rotary shaker at 150 r/min, 28 °C for 15 days. The cultures (50 L) were filtered through gauze to separate into mycelium and broth medium. The former was dried at 45 °C and ultrasonic extraction for 30 min with 95% ethanol, the extract was concentrated until no smell of alcohol and poured into the culture filtrate. Then the mixture was concentrated under reduced pressure to about 5 L and extracted for three times successively with ethyl acetate to generated in a crude residue (18 g). The residue was fractionated by MPLC with a MeOH/H_2O gradient system (0:100 to 100:0, v/v) to give fractions A–H. Fraction E was subjected to Sephadex LH-20 column chromatography with MeOH to give subfractions E1–E4. Subfraction E2 was purified by preparative HPLC (20–40% CH_3CN/H_2O, 30 min) to afford compound **6** (2.0 mg) and **9** (3.3 mg). Fraction F was subjected to Sephadex LH-20 column chromatography with MeOH to give subfractions F1–F4. Subfraction F1 was chromatographed on a silica gel, eluted with a system of Petroleum ether/Acetone (5:1) to give subfractions F1-1, F1-2. Subfraction F1-1 was purified by preparative HPLC (18–38% CH_3CN/H_2O, 25 min) to give compound **3** (4.3 mg). Subfraction F2 was purified by preparative HPLC (22-42% CH_3CN/H_2O, 25 min) to afford compounds **2** (10.2 mg), **7** (1.9 mg), and **10** (2.3 mg).

Fraction G was subjected to Sephadex LH-20 column chromatography with MeOH to give subfractions G1–G4. Subfractions G2 was chromatographed on a silica gel, eluted with a system of Petroleum ether/Acetone (8:1) to give subfractions G2-1, G2-2 and G2-3. Subfractions G2-2 and G2-3 were purified by preparative HPLC (25–45% CH_3CN/H_2O, 30 min) to afford compounds **4** (3.1 mg) and **5** (2.0 mg), respectively. Subfraction G3 was purified by preparative HPLC (26–46% CH_3CN/H_2O, 30 min) to produce compounds **1** (2.5 mg) and **8** (6.0 mg).

Compound 1: White powder; $[\alpha]_D^{23.7} = -34.7$ (c = 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 225 (3.5), 208 (3.4); IR (KBr) υ_{max}: 3433, 2925, 1750 cm^{-1}; ^1H and ^{13}C NMR data (Table S1); HR-ESI-MS (*m/z* 543.2518, $[M + Cl]^-$, calcd for $C_{31}H_{40}O_6Cl$, 543.2519).

4. Conclusion

A new steroid lactone aspergilolide, and nine known compounds were isolated from cultures of the endophytic fungus *Aspergillus sp*. MBL1612. The new compound showed no significant cytotoxicity against five cancer cell lines A549, MDA-MB-231, MCF-7 cells, KB and KB-VIN. All of the compounds were isolated from this genus for the first time, and the known compounds have revealed multiple bioactivity including antibacterial, antifungal, antitumor, etc (Wang et al. 1995; Zhao et al. 2006; Yin et al. 2009; Ren et al. 2011; Yang et al. 2013; Sun et al. 2014; Luo et al. 2017). The results of those active constituents isolated from the fungal strain *A.sp*. MBL1612 indirectly exhibit that endophytes have been considered as potential sources for various bioactive metabolites with intriguing structures, which could be useful drug candidates. However, the present study will conduct only as a comprehensively foreground basic research on involving in novel bioactive natural products by endophytes.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This project was supported by Key research and development project of Anhui Province (1704a0802145), Natural Science Key Research Program of Anhui Province University (KJ2017A294; KJ2016A853) and The Open Fund of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2017-KF03).

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