Nitrogen-Containing Compounds From Mangrove-Derived Fungus *Aspergillus* sp. 87

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**Abstract**

Nine nitrogen-containing compounds including 1 new alkaloid, aspergilamide A (1), and 8 known alkaloids and dipeptides, pseudotinin A (2), fumigaclavine C (3), isochaetominine (4), cyclo(L-Pro-L-tyr) (5), cyclo-trans-4-OH-(L)-Pro-(L)-Phe (6), brevianamide F (7), and spirotryprostatins A and B (8 and 9), were obtained from the mangrove-derived fungus *Aspergillus* sp. 87. Their structures were identified by extensive spectroscopic analyses. All compounds did not show significant antibacterial activities.

**Keywords**

mangrove-derived fungus, *Aspergillus*, nitrogen-containing compounds

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The *Aspergillus* genus is a diverse group of fungal species and is widespread in soil, plants, and animals.¹ It is well known for its industrial and commercial applications.² *Aspergillus* sp. can produce a variety of secondary metabolites with diverse novel structures and interesting biological activities.³ For example, cytotoxic quinone type compounds, variecolor quinones A and B, were isolated from the halotolerant fungal strain *Aspergillus variecolor* B-17;² antifouling benzylazaphilone derivative, aspergillose A, was obtained from a marine-derived fungus *Aspergillus* sp. isolated from the endophytic fungus *Aspergillus terreus*;³ and the anti-leishmanial butenolide derivative, terrenolide S, was isolated from the mangroves-derived fungus *Aspergillus terreus* from the roots of *Carthamus lanatus* (Asteraceae).⁶

Recently, our research group has focused on mangrove-derived fungi isolated from the South China Sea. *Aspergillus* sp. 87 was found to be an abundant producer of diverse secondary metabolites. The subsequent chemical investigation led to the discovery of 1 alkaloid, aspergilamide A (1), together with 8 known compounds, pseudotinin A (2), fumigaclavine C (3), isochaetominine (4), cyclo(L-Pro-L-tyr) (5), cyclo-trans-4-OH-(L)-Pro-(L)-Phe (6), brevianamide F (7), and spirotryprostatins A and B (8 and 9) (Figure 1). Herein, we report the isolation, structural elucidation, and bioactivity of these compounds.

**Results and Discussion**

The molecular formula of 1 was determined to be C₁₂H₁₉NO₅ from the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) (supplemental Figure S3) ion at m/z 256.11904 [M – H]⁻ (calcd for C₁₂H₁₈O₅N, 256.11905), indicating 4 degrees of unsaturation. The ¹H nuclear magnetic resonance (NMR) spectrum (supplemental Table S1) revealed the presence of 2 methyl groups (δ₁H 0.91 [2H, t, J = 7.4 Hz] and 1.29 [3H, d, J = 7.3 Hz]), 3 methylenes (δ₁H 1.34 [2H, m]; 1.60 [2H, m]; 4.11 [2H, tt, J = 6.5, 3.3 Hz]), 1 methine (δ₁H 4.43 [1H, q, J = 7.3 Hz]), 1 methoxy (δ₁H 3.76 [3H, s]), and 2 olefinic protons (δ₁H 6.68 [1H, d, J = 15.5 Hz] and 7.01 [1H, d, J = 15.5 Hz]). The ¹³C NMR data (supplemental Table S1) of 1 showed the presence of 12 carbons with the help of the heteronuclear single quantum coherence spectrum (supplemental Figure S8), 3 of which were non-protonated and belonging to either amide or ester carbonyl groups (δ₁c 167.2, 165.7, 173.8). The planar structure of 1 was identified by the ¹H-¹H correlation spectroscopy (COSY) (supplemental Figure S9) and

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heteronuclear multiple bond correlation (HMBC) (supplemental Figure S10) spectra. The key HMBC from methyl H-4 to carbonyl C-1 and C-2 and \(^1\)H-\(^1\)H COSY of H-5/H-6, as well as the chemical shifts of C-6 (δc 49.9) and C-7 (δc 173.8), established an alanine moiety. This was connected with a butyl group via an ester moiety, supported by the \(^{13}\)C NMR spectrum of 1, which showed signals for chemical shifts of 2 ester carbonyls C-1 (δc 167.2) and C-7 (δc 173.8), the secondary carbon of C-8 (δc 66.2), the 2D NMR spectrum of the 1H-1H COSY of H-8/H-9/H-10/H-11, and the HMBC from H-8 to C-7. A maleic acid moiety was assigned by HMBC from H-3 to C-1 and C-4 and H-2 to C-1 and C-4. The maleic acid and alanine were connected by an amide group according to the HMBC from H-6 to C-4. The remaining methoxy group was linked to carbonyl C-1 on the basis of the HMBC from –OCH\(_3\) to C-1. The geometry of the double bond was assigned as \(E\) on the basis of the classical trans vicinal coupling constant (\(J = 15.4\) Hz) between H-2 and H-3. The absolute configuration of 1 \([\alpha]^{28}_D = +17.35\) (c = 0.25, MeOH) was established as \(S\) compared with a known similar compound \((S,E)\)-benzyl-2-(but-2-enamino)-3-phenylpropionate \([\alpha]^{25}_D = +56.27\) (c = 1.5, CHCl\(_3\)) with the same optical activity sign.\(^7\) So, the structure of 1 was established as methyl \((S,E)-4-((1\text{-}\text{butoxy}-1\text{-}\text{oxopropan-2-yl})\text{amino})\)-4-oxobut-2-eneoate and named as aspergilamide A (Figure 2). Furthermore, using Marfey’s method, the absolute configurations of compound 1 were confirmed.\(^8\)

The known compounds were identified as pseurotin A (2),\(^9\) fumigaclavine C (3),\(^10\) isochaetominine (4),\(^11\) cyclo(L-Pro-L-tyr) (5),\(^12\) cyclo-trans-4-OH-(L)-Pro-(L)-Phe (6),\(^13\) brevianamide F (7)\(^14\) and spirotryprostatins A (8) and B (9)\(^15\) by comparison of their NMR data with literature values. All isolates 1-9 were evaluated for their antibacterial activities using 4 bacterial strains, \textit{Staphylococcus aureus} (ATCC 29213), \textit{Escherichia coli} (ATCC 25922), \textit{Pseudomonas aeruginosa} (ATCC 27853), and \textit{Acinetobacter baumannii} (ATCC 19606). All compounds were devoid of antibacterial activities against these 4 bacteria (minimum inhibitory concentration > 100 µM).

\section*{Experimental}

\subsection*{General Experimental Procedures}

Melting points were determined on a Fisher-Johns hot-stage apparatus and were uncorrected. Ultraviolet (UV) spectra were performed on a UV-116 spectrophotometer (Shimadzu, Beijing, China). Infrared (IR) spectra were recorded on a Fourier transformation infrared spectrometer coupled with an infrared microscope (EQUINOX 55, Bruker, Germany). The NMR data were measured on a Bruker Avance 400 MHz spectrometer.
spectrometer (Bruker BioSpin Corporation, Bellerica, MA, USA). Chemical shifts (δ) are given in ppm with reference to tetramethylsilane and coupling constants (J) in Hz. ESI-MS data were recorded on a TSQ Quantum Ultra mass spectrometer (TSQ Quantum Ultra, Thermofisher, Germany). HR-ESI-MS data were measured on an LTQ Orbitrap high-resolution mass spectrometer (LTQ Orbitrap Elite, Thermofisher, Germany). Column chromatography (CC) was performed on silica gel (200-300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia, Piscataway, NJ, USA). Precoated silica gel plates (Qingdao Huang Hai Chemical Group Co., Qingdao, China; G60, F-254) were used for thin-layer chromatography.

Fungal Material

The mangrove endophytic fungus 87 was isolated from the root of Aegiceras corniculata in Guangxi Shankou Mangrove Ecological National Nature Reserve, China. The strain was identified as Aspergillus sp. 87 using standard molecular biological protocols by deoxyribonucleic acid amplification and internal transcribed spacer sequencing. The sequence data obtained from the fungal strain have been deposited at GenBank, accession number KU306892. A voucher strain was deposited at the Institute of Marine Natural Products, School of Marine Sciences, Sun Yat-Sen University, China.

Fermentation, Extraction, and Isolation of Compounds 1-9

The fungus was grown in potato dextrose broth (PDB) liquid culture medium (potatoes, infusion from [300.0 g/L], dextrose 20.0 [g/L], sea salt [3 g/L]) in 98 Erlenmeyer flasks for 30 days at a constant temperature (28°C) in a static artificial climate incubator. The medium was divided into the fermented broth and fungal mycelia, which was exhaustively extracted using ethyl acetate (EtOAc) 3 times. The organic solvent was removed under reduced pressure to afford a crude extract (39.1 g). Then, the crude extract was divided into 5 fractions (A–E) by step-gradient elution using light petroleum/EtOAc (85:15, 70:30, 60:40, 50:50, 40:60).

Fraction B was applied to a RP-C18 column to give 2 (4 mg). Fraction C was subjected to Sephadex LH-20 chromatography and eluted with dichloromethane (CH2Cl2)–MeOH (1:1) and further separated by high-performance liquid chromatography (HPLC) using MeOH–H2O (70:30; 2.0 mL/min) to give 1 (13 mg, tR = 15 minutes). Fraction D was separated on an RP-C18 column to give subfractions D-1 and D-2. D-1 was purified by HPLC using MeOH–H2O (70:30; 2.0 mL/min) to give 3 (9 mg, tR = 21 minutes) and 4 (3 mg, tR = 23 minutes). D-2 was subjected to Sephadex LH-20 chromatography with CH2Cl2–MeOH (1:1) to give 5 (2.5 mg), 6 (2.7 mg), and 7 (3.5 mg). Fraction E was separated by HPLC using MeOH–H2O (60:40; 2.0 mL/min) to give 8 (3 mg, tR = 18 minutes) and 9 (2 mg, tR = 21 minutes).

Aspergilamide A (1): [α]D28 = +17.35 (c = 0.25, MeOH); colorless oil, UV (MeOH) λmax: 214 (3.82) and 250 (3.65) nm (supplemental Figure S1); IR (neat) νmax: 3300, 2959, 2874, 1731, 1667, 1544, 1452, 1349, 1309, 1169, 1087, 1056, 978, 773, and 671 cm−1 (supplemental Figure S2); HR-ESI-MS (m/z 256.11904 [M − H]+, calcld for 256.11905, C12H16NO5); 1H NMR, 13C NMR, Dept-90 and Dept-135 (supplemental Figures S1-11).

Bioactivity Assay

Antibacterial activities were evaluated by the conventional broth dilution assay as described previously. Four bacterial strains, S. aureus (ATCC 29213), E. coli (ATCC 25922), P. aeruginosa (ATCC 27853), and A. baumannii (ATCC 19606), were used.

Marfey’s Derivatization and HPLC Analysis

Compound 1 (0.5 mg) was treated with 6 M hydrochloric acid (HCl; 500 µL) at 115°C for 18 hours. The hydrolyzed products were concentrated to dryness under a stream of nitrogen and dissolved in 100 µL of H2O. Then, 100 µL of 1% (w/v) 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide in acetonitrile and 50 µL of sodium bicarbonate (1 N) were added, and the mixture was incubated (1 hour at 40°C) and stopped by addition of 10 µL of HCl (2 M). The l-alanine was treated with FDAA for the research, authorship, and/or publication of this article: This article has been co-authored by two or more individuals. The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This would mean that the author(s) received financial support for the research, authorship, and/or publication of this article: This would mean that the author(s) received financial support for the research, authorship, and/or publication of this article.

Conclusion

In summary, 9 secondary metabolites, including 1 new alkaloid, aspergilamide A (1), and 8 known alkaloids and dipeptides, peumorin A (2), fumigaclavine C (3), isocheatomine (4), cyclo(L-Pro-L-tyr) (5), cyclo-trans-4-OH-(L)-Pro-(L)-Phe (6), brevianamide F (7), and spirotryprostatins A (8) and B (9), were isolated and identified from the mangrove-derived fungus Aspergillus sp. 87 in rice solid-substrate fermentation.

Declaration of Conflicting Interests

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