Trichoderlates A and B, Two Bioactive Metal Complexes of Hydroxyaspergillic Acid from the Marine-derived Fungus *Trichoderma erinaceum* F1-1

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Abstract

Two new metal complexes of hydroxyaspergillic acid, trichoderlates A (1) and B (2), and five previously reported compounds (3-7) were obtained from this culture. This is the first report about metal complexes of hydroxyaspergillic acid from the genus *Trichoderma*. Trichoderlates A (1) and B (2) exhibited significant cytotoxicities against MDA-MB-435 human melanocytic cancer cells with IC50 values of 3.3 and 9.5 μM, respectively. Trichoderlate A (1) also showed significant cytotoxic activity against A549 adenocarcinomic human alveolar basal epithelial cells with IC50 value of 6.1 μM.

**Keywords:** Metal complexes; Cytotoxic activities; Marine-derived fungus; *Trichoderma erinaceum*

**Introduction**

Secondary metabolites (SMs), as chemical entities of defense against biological warfare, were produced by organisms in answer to environmental stimuli [1]. Those SMs isolated from plants, bacteria and fungi provide a diverse and unique source of candidates for drugs discovery. SMs derived from the marine fungi are well known for showing extraordinary potential biological activity due to the specific habitat environment relative to terrestrial ones. For instance, pyrazin-2(1H)-one-derivatives derived from amino acids were well known for showing significant cytotoxicities against various cancer cell lines and strong toxicity towards brine shrimp [2-7]. By thoroughly literature survey, less than 40 natural pyrazin-2(1H)-one derivative in the literatures have been reported, and only five metal complexes of pyrazin-2(1H)-one derivative have been isolated from *A. ochraceus* Wilh., *A. flavus* SCGAP0093, *Aspergillus* sp. 16-02-1, the co-culture of *P. citrinum* and *A. ochraceus* SCGAF0093 [11]. Despite these findings, the potential of marine fungal secondary metabolites, including pyrazin-2(1H)-one derivative, have been under explored [9].

The genus *Trichoderma*, which is widely spread in nature, is commonly considered a biocontrol agent in agriculture. By 2008, more than 180 metabolites have been isolated from species belonging to this genus [10]. And a wide range of bioactivities of these compounds, including antibacterial, antifungal, antiviral activities, have been reported. In our previous work, two harziane-related diterpenoids were discovered from the glucose-peptone-yeast (GPY) medium of the marine-derived fungus *T. erinaceum* F1-1 associated with the Sea Star *A. planci* [11].

In our continued research project, the *Trichoderma erinaceum* F1-1 was cultured in potato dextrose broth (PDB) medium. Two new metal complexes of hydroxyaspergillic acid, trichoderlate A (1) and trichoderlate B (2), and five previously reported compounds (3-7) were obtained from this culture. This is the first report about metal complexes of hydroxyaspergillic acid from *Trichoderma* genus. Herein, we describe the isolation, structure characterization, and bioactivities of compounds 1-7.

**Materials and Methods**

**General experimental procedures**

The NMR data were obtained from Bruker Avance III 500HD and 400 spectrometer made in Bruker Bio Spin AG. Referencing the signals of CDCl3 (δH 7.26 and δC 144.0), Methanol-d4 (δH 3.31 and δC 49.0) to obtain the relative NMR chemical shifts. HRESIMS data were measured on Thermo MAT95XP EI high-resolution mass spectrometers made in Thermo Fisher Scientific In Preparative HPLC and column chromatography was performed adopting a Shim-pack PRC-ODS HPLC column (250 x 20 mm) made in Shimadzu Corporation, silica...
gel (SiO₂, 200-300 mesh) made in Qingdao Marine Chemical Inc., and Sephadex LH-20 (green herbs) made in Beijing. UV, IR, and Optical rotations data were recorded on a UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Nakagyo-Ku, Kyoto, Japan), a Frontier FT-IR spectrophotometer (PerkinElmer Inc., Waltham, MA, USA), and an Anton paar MCP500 polarimeter, respectively.

Fungal strain and culture method

\textit{T. erinaceum} F1-1 was purified from the inner tissue of the sea star \textit{A. planci}, which habited in Hainan Sanya National Coral Reef Reserve, China. Based on morphology and the results of the ITS regions of rDNA, the genus was identified. And its sequence data have been deposited in GenBank with the accession number KF999808. The PDB fermentation medium contains potatoes 200 g, dextrose 20 g, seawater 1 L and pH 7.5. In a sterile condition, transferring the mycelia to sterilized 200 mL of the PDB liquid medium in 500 mL Erlenmeyer flasks. After incubating 40 d at 28°C, 100 L culture broth were filtered and extracted four times with EtOAc, and then were concentrated to afford a crude extract 20 g.

Extraction and isolation

The extract (20 g) was fractionated on the Si gel column with a petroleum-EtOAc gradient (100:0-100) and then EtOAc-MeOH gradient (100:0-1:100) to obtain 12 fractions (Fr.1-Fr.12). Fr.4 was separated into six fractions (Fr.4.1-Fr.4.6) by the Sephadex LH-20 (MeOH), Fr.4.4-2 was further fractionated using the preparative HPLC with MeOH-H₂O (30:70-100:0) to obtain 1 (30.0 mg), 2 (16.0 mg) and 3 (5.2 mg). 5 (23.0 mg) and 7 (4.3 mg) were yielded from Fr.5 by the column chromatography on the Si gel with petroleum-EtOAc (100:0-1:100). Compound 4 (8.7 mg) was purified from Fr. 7 by repeated CC on the Si gel eluting with petroleum-EtOAc (30.70). 6 (2.0 mg) was purified from Fr.9 by the Sephadex LH-20 (MeOH).

\textbf{Trichoderlate A (1)}: A red solid; UV (MeCN) \( \lambda_{\text{max}} (\log \epsilon) \): 312 (2.36) nm, 255 (4.50) nm; \([\alpha]_{20}^\circ +40.0 (c 0.5, \text{MeOH})\); IR \( \nu_{\text{max}} \): 3408, 2979, 1590, 1531, 1497, 1418, 1169 cm\(^{-1}\); \(1^H\) and \(1^3C\) NMR data (Table 1) HRESIMS [M+H\(^+\)] \( ions \) at \( m/z \) 774.3618 (calculated (calcd) for \( C_{36}H_{58}AlN_6O_9, 774.3615 \)). A dozen of carbons (Table 1) were placed in C-6 (\( \delta \) 141.7) (Figure 4). The comparison of spectroscopic data and the molecular formula showed that 1 was structurally similar to hydroxyaspergillic acid (3) [15]. Furthermore, the HMBC correlation between H-7 (\( \delta \) 2.64, m), H-8 (\( \delta \) 2.01, m) and H-9 (\( \delta \) 0.83, m) indicated the existence of the segment -CH- (CH₃)₂. According to the HMBC correlation between H-7 (\( \delta \) 2.64, m) and C-3 (\( \delta \) 152.5), the segment -CH₂-CH- (CH₃)₂ was placed in the C-3. In addition, the COSY correlation of H-12 (\( \delta \) 1.93, m) and H-13 (\( \delta \) 0.80, m) and HMBC correlations between H-12 (\( \delta \) 1.93, m), H-13 (\( \delta \) 0.80, m), H-14 (\( \delta \) 1.58, s) and C-11 (\( \delta \) 73.5) revealed the existence of the attachment -C (OH) (CH₃)₂ (CH₂)₃(C₆H₅). Furthermore, the HMBC correlations between H-5 (\( \delta \) 7.75, s) and C-11 (\( \delta \) 73.5), C-6 (\( \delta \) 141.7) indicated that the substructure -C (OH) (CH₃)₂ (CH₂)₃(C₆H₅) was placed in C-6 (\( \delta \) 141.7) (Figure 4).

\textbf{Optical rotation calculations}

MOE software in the MMFF94 force field was used to the conformational analysis of compounds [12]. The optical rotations were calculated using time-dependent density functional theory (TDDFT) [13]. We employed the B3LYP/6-311+G (d) level for optimizing the conformers and calculating optical rotations at \( \lambda = 589.3 \) nm in methanol (Figures 2 and 3).

\textbf{Bioassay}

\textbf{Cytotoxic assay}: Compounds 1-7 were evaluated against human tumor cell line MDA-MB-435 and A549 by MTT method described in the literature [14]. Cisplatin was used as positive control (IC₅₀ of cisplatin is 35.1 \( \mu \)M for A549 cell line and 8.5 \( \mu \)M for MDA-MB-435 cell line).

\textbf{Results and Discussion}

\textbf{Structural elucidation}

\textbf{Trichoderlate A (1)} was obtained as a red solid and gave a molecular formula of \( C_{36}H_{58}AlN_6O_9 \) by HRESIMS ion at \( m/z \) [M+H\(^+\)] \( = 774.3618 \) (calculated (calcd) for \( C_{36}H_{58}AlN_6O_9, 774.3615 \)). A dozen of carbons (Table 1) were classified as four methyl, two methylene, two methines, and four non-protonated carbons. COSY correlations between H-7 (\( \delta \) 2.64, m), H-8 (\( \delta \) 2.01, m) and H-9 (\( \delta \) 0.83, m), as well as between H-8 (\( \delta \) 2.01, m) and H-10 (\( \delta \) 0.83, m) indicated the existence of the segment -CH₂- (CH₃)₂. According to the HMBC correlation between H-7 (\( \delta \) 2.64, m) and C-3 (\( \delta \) 150.7), the segment -CH₂-CH- (CH₃)₂ was placed in the C-3. In addition, the COSY correlation of H-12 (\( \delta \) 1.93, m) and H-13 (\( \delta \) 0.80, m) and HMBC correlations between H-12 (\( \delta \) 1.93, m), H-13 (\( \delta \) 0.80, m), H-14 (\( \delta \) 1.58, s) and C-11 (\( \delta \) 73.5) revealed the existence of the attachment -C (OH) (CH₃)₂ (CH₂)₃(C₆H₅) (-). Furthermore, the HMBC correlations between H-5 (\( \delta \) 7.75, s) and C-11 (\( \delta \) 73.5), C-6 (\( \delta \) 141.7) indicated that the substructure -C (OH) (CH₃)₂ (CH₂)₃(C₆H₅) was placed in C-6 (\( \delta \) 141.7) (Figure 4). The comparison of spectroscopic data and the molecular formula showed that 1 was structurally similar to hydroxyaspergillic acid (3) [15]. Furthermore, alkali hydrolysis of 1 gave 3 and Fe (OH)₃ sediment [4], indicating 1 was an iron complex of 3. Based on the molecular formula, it was assured that trichoderlate A (1) was a complex consisting of three molecules of hydroxyaspergillic acid (3) and one iron atom. For the known metal complexes of pyrazin-2(1H)-one derivative, aluminium neo hydroxy aspergilinand...
The chemical structures of the known compounds hydroxyaspergillic acid (3) [15], 4-(1-hydroxy-1-methylpropyl)-2-isobutyl-pyrazin-2(1H)-one (4) [16], dichotomocej A (5) [17] and fenestin A (6) [18], linoleic acid (7) [19] were confirmed respectively by comparing their spectroscopic data of relevant references.

**Biological activity**

Compounds 1-7 were evaluated against human tumor cell line MDA-MB-435 and A549 by MTT method described in the literature [14]. Trichoderlate A (1), trichoderlate B (2) and hydroxyaspergillic acid (3) exhibited cytotoxic activities against MDA-MB-435 human melanocyte cancer cells with IC₅₀ values of 3.3, 9.5, 49.9 μM, respectively. Trichoderlates A (1) and B (2) also showed cytotoxic activities against A549 adenocarcinomic human alveolar basal epithelial cells with IC₅₀ values of 6.1, 15.6 μM.
Conclusion

Overall, two new metal complexes of hydroxyaspergillic acid, trichoderlates A (1) and B (2) were afforded from the PDB medium culture of *Trichoderma erinaceum* F1-1. And five known compounds (3-7) were also identified. Furthermore, this is the first report about metal complexes of hydroxyaspergillic acid from the genus *Trichoderma*. How the metals iron and aluminium are involved in the biosynthesis of the fungus, and how the enzymes catalyze the biosynthesis of complexes remain unknown.

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Conflicts of Interest

The authors declare no conflict of interest.

References