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# Aspergiolide A, a novel anthraquinone derivative with naphtho[1,2,3-*de*]chromene-2,7-dione skeleton isolated from a marine-derived fungus *Aspergillus glaucus*

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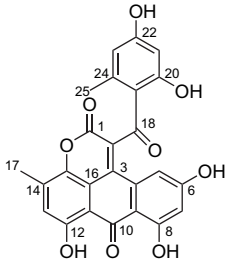
**Abstract**—Aspergiolide A (**1**), an anthraquinone derivative with naphtho[1,2,3-*de*]chromene-2,7-dione skeleton, has been isolated from cultures of a marine-derived fungus *Aspergillus glaucus*. The structure and stereochemistry of **1** was determined by NMR and X-ray diffraction analyses. Compound **1** showed selective cytotoxicities against A-549, HL-60, BEL-7402, and P388 cell lines.


## 1. Introduction

Marine filamentous fungi have been proved to be a fertile source of bioactive compounds.[1] In our continuous search for anti-tumor compounds from marine-derived microorganisms, a fungus, authenticated as *Aspergillus glaucus*, was obtained from the marine sediment around the mangrove roots collected in Fujian province of China. Its extract exhibited cytotoxicity against K562 and P388 cell lines. The isolation work of the active compounds on this fungus led to the finding of a novel anthraquinone derivative with naphtho[1,2,3-*de*]chromene-2,7-dione skeleton, namely, aspergiolide A (**1**). In this paper, we describe the isolation, structure elucidation, and cytotoxicities against A-549, HL-60, BEL-7402, and P388 cell lines of **1**.




**1**

*Keywords*: *Aspergillus glaucus*; Naphtho[1,2,3-*de*]chromene-2,7-dione; Aspergiolide; Microorganism; Fungus.
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## 2. Results and discussion

*A. glaucus* HB1-19 was cultured in liquid medium for nine days and the metabolites were extracted with EtOAc. The crude extracts were chromatographed on silica gel columns and subjected to extensive reversed-phase preparative HPLC to give pure compound **1**.

Compound **1** was obtained as a red needle. Its molecular formula $C_{25}H_{16}O_9$ was determined by HRESI-MS (*m/z*: 459.0725 $[M-H]^-$, calcd 459.0716), indicating 18 degrees of unsaturation. Its IR spectrum exhibited strong absorptions at 3443 and 1649 $cm^{-1}$, indicative of hydroxyl and conjugated carbonyl groups. The UV spectrum showed absorptions at $\lambda_{max}$ (log $\varepsilon$) 203 (4.25), 235 (4.19), 307 (3.90), and 431 (3.61) nm in MeOH implying the presence of large conjugated system. The $^1H$ NMR data of **1** (Table 1) showed characteristic signals of two methyl groups at $\delta_H$ 2.46 (3H, s, Me-25) and 2.56 (3H, s, Me-17), two pairs of *meta*-coupled aromatic protons at $\delta_H$ 6.11 (1H, s, H-23) and 6.27 (1H, s, H-21), 6.94 (1H, s, H-7), and 6.50 (1H, s, H-5), which were mutually coupled in the $^1H$–$^1H$ COSY spectrum. The $^{13}C$ NMR and DEPT data for **1** displayed 21 carbon signals comprising 2 methyls, 5 methines, and 14 quaternary carbons (Table 1).

Analysis of the HMBC correlations of H-5/C-3, C-6, C-7; H-7/C-5, C-6, C-8, C-9; H-13/C-11, C-15; H-17/C-13, C-14, C-15; HO-8/C-8, C-9 combined with comparison of the $^1H$ NMR and $^{13}C$ NMR data with those of catenarin and rubrocristin, two known anthraquinones,[2] could lead to the 1,3,8-trihydroxy-6-methyl-9-anthrone moiety (Fig. 1).

**Table 1**. $^{1}H$ and $^{13}C$ NMR, COSY, and HMBC data of compound **1** (600, 150, and 600 MHz, DMSO-$d_6$, TMS, $\delta$ ppm)

| No. | $\delta_H$ ($J$ in hertz) | $\delta_C$ | $^{1}H$–$^{1}H$ COSY | HMBC (H→C) |
|---|---|---|---|---|
| 1 | | 158.9 s[a] | | |
| 2 | | 145.4 s | | |
| 3 | | 107.8 s | | |
| 4 | | 133.9 s | | |
| 5 | 6.94 s | 110.9 d | 7 | 3,6,7 |
| 6 | | 164.8 s | | |
| 7 | 6.50 s | 105.4 d | 5 | 5,6,8,9 |
| 8 | | 165.6 s | | |
| 9 | | 108.0 s | | |
| 10 | | 187.7 s | | |
| 11 | | 107.8 s | | |
| 12 | | 162.9 s | | |
| 13 | 7.25 s | 122.1 d | 17 | 11,15 |
| 14 | | 136.2 s | | |
| 15 | | 142.6 s | | |
| 16 | | 115.9 s | | |
| 17 | 2.46 s | 16.0 q | 13 | 13,14,15 |
| 18 | | 190.8 s | | |
| 19 | | 114.9 s | | |
| 20 | | 164.4 s | | |
| 21 | 6.11 s | 100.7 d | 23 | |
| 22 | | 159.0 s[a] | | |
| 23 | 6.27 s | 112.0 d | 21 | 19,21 |
| 24 | | 145.3 s | | |
| 25 | 2.56 s | 23.2 q | | |
| OH-6 | 10.52 s[b] | | | |
| OH-8 | 12.91 s | | | 8,9 |
| OH-12 | 12.39 s | | | |
| OH-20 | 11.30 s | | | |
| OH-22 | 10.39 s[b] | | | |

[a] $^{13}C$ NMR data can be exchanged.
[b] $^{1}H$ NMR data can be exchanged.

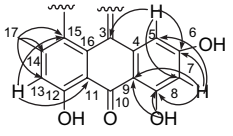


**Figure 1**. 9-Anthrone partial structure in **1** and its HMBC correlations.

However, the structure of **1** could not be easily deduced because there was no correlation of H-21 with any carbon and no correlation of any hydrogen with C-1, C-2, and C-18 in the HMBC spectrum.

Fortunately, red crystals of **1** could be obtained for X-ray crystallographic analysis, which confirmed the structure of **1** (Fig. 2).

Compound **1** was preliminarily tested for its cytotoxic activities using P388, HL-60 cell lines by the MTT method[3] and BEL-7402, A-549 cell lines by the SRB method[4] according to the procedures previously reported. Compound **1** showed selective cytotoxicities against A-549, HL-60, BEL-7402, and P388 cell lines with $IC_{50}$ values of 0.13, 0.28, 7.5, and 35.0 μM, respectively.

*A. glaucus* species were reported to produce several active anthraquinones by polyketide pathway,[5] such as emodin, erythroglaucin, physcion, physcion-9-anthrone, questin, catenarin, and rubrocristin.[6] Aspergiolide A is the first example with naphtho[1,2,3-*de*]chromene-2,7-dione skeleton isolated from a natural source. Careful analysis of the structure

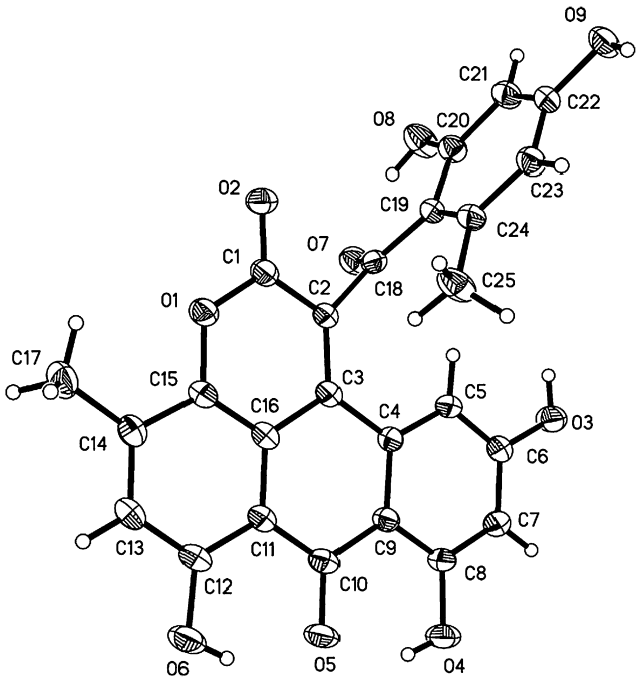


**Figure 2**. X-ray crystal structure of **1**.

could lead to the postulated biosynthetic pathway of **1** with an anthraquinone precursor, catenarin, which was also isolated from this fungus, and a supposed polyketide precursor **2**, which was probably formed by one acetyl-CoA unit and four malonyl-CoA units. The aldol condensation then took place between catenarin and **2** that served as a nucleophile, and the formed intermediate further underwent intramolecular esterification between the carboxyl and the hydroxyl groups to yield **1** (Fig. 3).

A few compounds of 9-anthrone lactone, such as 6-hydroxynaphtho[1,2,3-*de*]chromene-2,7-dione, 2,7-dioxo-2,7-dihydronaphtho[1,2,3-*de*]chromene-1-carbonitrile, and 1-phenylnaphtho[1,2,3-*de*]chromene-2,7-dione, were reported as the synthetic intermediates[7] but no data about the bioactivity of this type were reported. We have first reported the structure and cytotoxic activities of aspergiolide A.

## 3. Experimental

### 3.1. General

Melting points were measured using a Yanaco MP-500D micro-melting point apparatus and were uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on Beckman DU® 640 spectrophotometer. IR spectra were taken on a NICOLET NEXUS 470 spectrophotometer in KBr discs. $^{1}H$, $^{13}C$ NMR and DEPT spectra, and 2D NMR were recorded on a JEOL Eclips-600 spectrometer using TMS as internal standard and chemical shifts were recorded as $\delta$ values. ESI-MS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pak ODS-A, 10×250 mm, 5 μm, 4 mL/min].

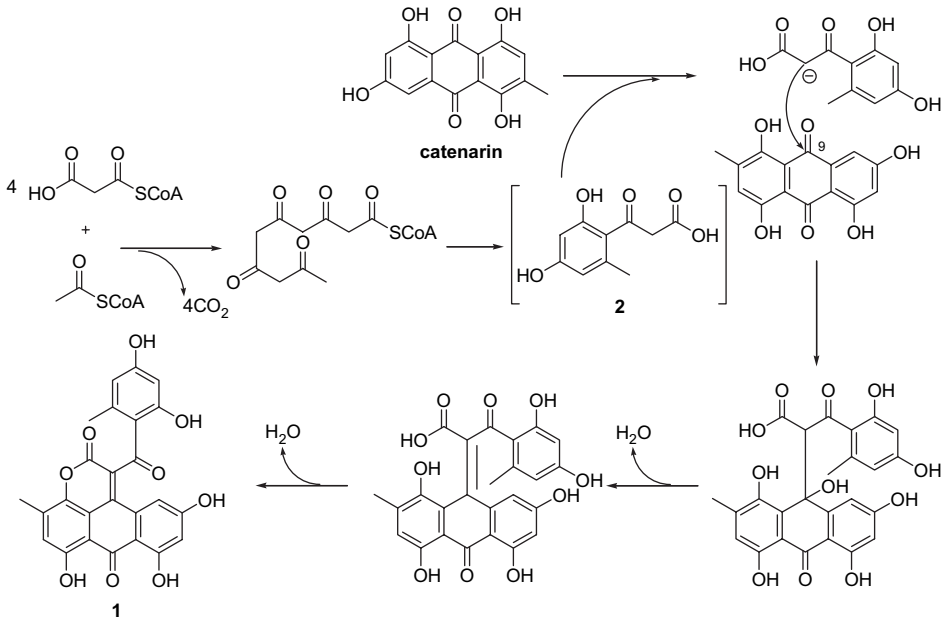


**Figure 3**. Postulated biosynthetic pathway of **1**.

### 3.2. Culture of *A. glaucus*, strain HB1-19, extraction and isolation of aspergiolide A (1)

The producing strain was preserved in China Center for Type Culture Collection (patent depository number: CCTCC M 206022). Fermentation was carried out as follows. Spores were directly inoculated into 500 mL Erlenmeyer flasks containing 100 mL fermentation media (mannitol 20 g, maltose 20 g, glucose 10 g, monosodium glutamate 10 g, $KH_2PO_4$ 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, yeast extract 3 g, and corn steep liquor 1 g, dissolved in 1 L sea water, pH 6.5). The flasks were incubated on a rotatory shaker at 165 rpm at 28 °C. After nine days of cultivation, 15 L of whole broth was filtered through cheesecloth to separate the broth supernatant and mycelia. The former was extracted with ethyl acetate, while the latter was extracted with acetone. The acetone extract was evaporated under reduced pressure to afford an aqueous solution, and then extracted with ethyl acetate. The two ethyl acetate extracts were combined and concentrated in vacuo to give a crude gum (30 g). The crude gum was subjected to silica gel column chromatography ($CHCl_3$/MeOH, v/v, gradient) and the active fraction Fr. 4 was recrystallized to afford compound **1** (20 mg).

### 3.3. X-ray diffraction analysis of aspergiolide A (1)

X-ray crystal structure analysis of compound **1**: red block crystal of $C_{25}H_{16}O_9$. Space group $P2_1/c$, $a$=19.168(4), $b$=7.6322(15), $c$=14.576(3) Å, $\beta$=111.534(4)°, $V$=1983.5(7) Å$^3$, $Z$=4, crystal size 0.20×0.17×0.08 mm$^3$. A total of 4845 unique reflections ($2\theta$<56.64°) were collected using graphite monochromated Mo Kα ($\lambda$=0.71073 Å) on a CCD area detector diffractometer. The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques (SHELXS-97). The final cycle of full-matrix least squares refinement was based on 4845 unique reflections ($2\theta$<56.64°) and 307 variable parameters and converged with unweighted and weighted agreement factors of $R$=0.126, $R_w$=0.171, and $R_1$=0.052 for $I>2.0\sigma(I)$ data. Crystallographic data (excluding structure factors) for structure **1** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 614797. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

### 3.4. Biological assays

Cytotoxic activity was evaluated using P388, HL-60 cell lines by the MTT method[3] and BEL-7402, A-549 cell lines by the SRB method.[4]

In MTT assay, the cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% $CO_2$ and 95% air at 37 °C. Those cell suspensions (200 μL) at a density of $5\times10^4$ cell mL$^{-1}$ were placed in 96 well microtiter plates and incubated for 24 h under the above condition. Then 2 μL of the test compound solutions (in MeOH) at different concentrations was added to each well and further incubated for 72 h under the same condition. The MTT solution (20 μL, 5 mg/mL in IPMI-1640 medium) was added to each well and incubated for 4 h. An old medium (150 μL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a SPECTRA MAX PLUS plate reader at 540 nm.

In SRB assay, 200 μL of the cell suspensions was placed in 96 cell plates at a density of $2\times10^5$ cell mL$^{-1}$. Then 2 μL of the test compound solutions (in MeOH) at different concentrations was added to each well and the culture was further incubated for 24 h. Following drug exposure, the cells were fixed with 12% trichloroacetic acid and the cell layer was stained with 0.4% SRB. The absorbance of

SRB solution was measured at 515 nm. Dose response curves were generated and the $IC_{50}$ values, the concentration of compound required to inhibit cell proliferation by 50%, were calculated from the linear portion of log dose response curves.

*Aspergiolide A*: red crystals ($CHCl_3$/MeOH=20:1); HRESI-MS $[M-H]^-$ *m*/*z* 459.0725, calcd for $C_{25}H_{15}O_9$, 459.0716; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 203 (4.25), 235 (4.19), 307 (3.90), 431 (3.61) nm; IR (KBr) $\nu_{max}$ 3443, 2955, 2854, 2955, 2925, 2854, 1649, 1632, 1622, 1557, 1540, 1507, 1457, 1441, 1417, 1393, 1260, 1205, 1164, 1098, 1028, and 961 $cm^{-1}$.

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