A Cytotoxic Xanthone Dimer from the Entomopathogenic Fungus *Aschersonia* sp. BCC 8401

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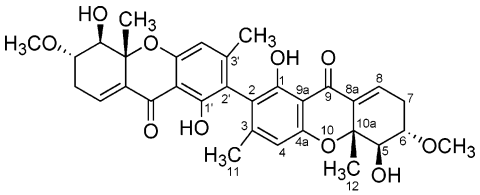
Ascherxanthone A (**1**), a novel symmetrical tetrahydroxanthone dimer, was isolated from the entomopathogenic fungus *Aschersonia* sp. BCC 8401. The structure of **1** was elucidated by spectroscopic analysis, especially 2D-NMR. Compound **1** exhibited activity against *Plasmodium falciparum* K1 with an IC_{50} value of 0.20 μg/mL, but it also showed cytotoxic activities against Vero cells and three tumor cell lines.

In our search for novel bioactive compounds from insect pathogenic fungi,[1,2] we observed significant antimalarial activity (IC_{50} 0.30 μg/mL) in the extract from a culture of *Aschersonia* sp. BCC 8401, which was collected on a Homoptera-scale insect. Investigation of the chemical constituents was undertaken, which led to the isolation of a novel tetrahydroxanthone dimer, ascherxanthone A (**1**). There have been few reports on secondary metabolites from the genus *Aschersonia*: 3β,15α,22-trihydroxyhopane (a triterpene) from *A. aleyroidis*,[3] its 3β-*O*-acetate from *A. tubulata* BCC 1785,[4] and destruxins A_4 and A_5 (cyclodepsipeptides) from an *Aschersonia* species.[5]



1

Ascherxanthone A (**1**) was isolated as a yellow powder from the MeOH extract of mycelia of *Aschersonia* sp. BCC 8401. The molecular formula of this compound was estimated to be $C_{32}H_{34}O_{10}$ by HRMS. The presence of only 16 carbon signals in the ^{13}C NMR spectrum indicated a symmetric, homodimer structure. Analysis of ^1H and ^{13}C NMR, DEPT, and HMQC spectra revealed that half of the molecule, $C_{16}H_{17}O_5$, possessed a conjugated ketone carbonyl (δ_C 185.3), seven quaternary carbons (δ_C 160.5, 158.3, 150.8, 135.6, 115.9, 105.3, and 82.3), two sp^2 methines (δ_C 133.3, δ_H 6.87; δ_C 109.3, δ_H 6.61), two oxymethines (δ_C 75.9, δ_H 4.13; δ_C 76.9, δ_H 3.48), one methylene (δ_C 30.9, δ_H 2.97 and 2.33), two methyl groups attached to quaternary carbons (δ_C 21.0, δ_H 2.10; δ_C 20.0, δ_H 1.59), and one methoxyl (δ_C 57.3, δ_H 3.52), and also observed was a proton signal of a chelated phenolic hydroxyl (δ_H 12.40). COSY correlations revealed the connectivity of C-5 to C-8, and the tetrahydroxanthone structure was established on the basis of the HMBC correlations. Intense correlation from the chelated phenolic proton (O*H*-1; δ_H 12.40) to the quaternary carbon at δ_C 115.9 placed this carbon at C-2; hence, this compound was a C-2−C-2′ dimer. In addition to the standard two- and three-bond correlations, a weak four-bond correlation from an aromatic proton (H-4, δ_H 6.61) to a ketone carbonyl (C-9, δ_C 185.3) was observed.

The relative configuration at C-6, C-5, and C-10a in ascherxanthone A (**1**) was deduced from the ^1H NMR and NOESY data. The ring fusion necessitated the pseudoaxial orientation of the methyl group adjacent to C-10a. In the NOESY spectrum of **1**, a correlation between the C-12 methyl protons (δ_H 1.59) and H-6 was observed. The large coupling constant between H-5 and H-6, J = 10.2 Hz, indicated the antiperiplanar relationship of these protons (both pseudoaxial); therefore the hydroxyl group (on C-5) and the methoxyl group (on C-6) should be pseudoequatorial. Unfortunately, attempts to prepare α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of ascherxanthone A (**1**) were not successful, and the absolute configuration of **1** remains questionable.

The structure of ascherxanthone A (**1**), the C-2−C-2′ dimer of 5,6,8a,10a-tetrahydroxanthone, is related to the secalonic acids[6] and substances TMC315A1 and TMC315A2, which were recently isolated from *Ceuthospora* sp. TMC1678 and claimed in a Japanese patent as RANKL (receptor activator of NF-κB ligand) antagonists.[7] A remarkable structural difference between **1** and other secalonic acid derivatives is the lack of hydroxyl groups at C-8 and C-8′ in **1**.

Ascherxanthone A (**1**) exhibited significant activity against *Plasmodium falciparum* K1 with an IC_{50} value of 0.20 μg/mL; however, it also showed cytotoxicity to Vero cells (IC_{50} 0.80 μg/mL) and three cancer cell lines, KB, BC, and NCI-H187, with respective IC_{50} values of 1.7, 1.7, and 0.16 μg/mL. Related compounds, secalonic acids, are reported to exhibit various biological activities such as cytostatic activity (mouse leukemia L1210 cells),[8] phlogistic activity,[9] inhibition of protein kinase C and cyclic AMP-dependent protein kinase,[10] and as toxicity to mice.[11]

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1030 polarimeter. UV spectra were recorded on a Varian CARY 1E UV−visible spectrophotometer. FT-IR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were taken on a Bruker AV500D spectrometer. ESI-TOF mass spectra were measured with a Micromass LCT mass spectrometer.

Fungal Material. *Aschersonia* sp. was collected on a Homoptera-scale insect, at Khao Yai National Park, central

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Thailand. This fungus was deposited at the BIOTEC Culture Collection (BCC) as BCC 8401.

Fermentation, Extraction, and Isolation. The fungus BCC 8401 was maintained on potato dextrose agar at 25 °C, after which the mycelium was cut into pieces (1 × 1 cm) and inoculated in two 250 mL Erlenmeyer flasks, each containing 25 mL of Difco potato dextrose broth (PDB). After incubation at 25 °C for 8 days on a rotary shaker (200 rpm), these primary seed cultures were transferred into two 1000 mL Erlenmeyer flasks, each containing 250 mL of PDB, and incubated at 25 °C for 8 days on a rotary shaker (200 rpm). Each 25 mL portion of the secondary seed cultures was transferred into twenty 1000 mL Erlenmeyer flasks, each containing 250 mL of M102 medium (composition: sucrose 30.0 g, malt extract 20.0 g, bacto-peptone 2.0 g, yeast extract 1.0 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, and KH_2PO_4 0.5 g, in 1000 mL of distilled water), and fermentation was carried out at 25 °C for 28 days on rotary shakers. Then, the cultures were filtered, and the residual mycelial cakes were extracted at rt with MeOH (1000 mL) for 2 days and filtered. To the filtrate was added H_2O (25 mL), and the mixture was washed with hexane (800 mL). The aqueous MeOH layer was concentrated under reduced pressure. The residue was dissolved in EtOAc (1000 mL) and washed with H_2O (100 mL), and the organic layer was concentrated under reduced pressure, leaving a deep yellow solid (3.1 g). This extract was triturated in MeOH (5 mL) and filtered by suction. The residual solid (1.52 g) was mainly composed of zeorin (known hopane triterpene). The filtrate (dry weight, 1.35 g) was subjected to a Sephadex LH20 column (4.0 × 30 cm; elution with MeOH). Fractions containing yellow pigment were combined (296 mg) and subjected to silica gel column chromatography (2.5 × 20 cm; EtOAc/CH_2Cl_2, gradient elution from 0:100 to 30:70) to obtain a yellow solid (**1**; 108 mg; R_f 0.35, MeOH/CH_2Cl_2, 5:95). Ascherxanthone A (**1**) was further purified by recrystallization in CH_2Cl_2/hexane.

Ascherxanthone A (1): yellow solid; mp 240−244 °C; $[\alpha]^{24}_D$ +227° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.78), 221 sh (4.60), 258 sh (4.34), 308 (4.61), 381 (3.92) nm; IR (KBr) ν_{max} 3482, 2935, 1632, 1604, 1407, 1264, 1091, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 12.40 (2H, s, 1-O*H* and 1′-O*H*), 6.87 (2H, dd, J = 5.0, 2.8 Hz, H-8 and H-8′), 6.61 (2H, s, H-4 and H-4′), 4.13 (2H, d, J = 10.3 Hz, H-5 and H-5′), 3.52 (6H, s, 6-OCH_3 and 6′-OCH_3), 3.48 (2H, ddd, J = 10.3, 9.2, 6.2 Hz, H-6 and H-6′), 2.97 (2H, ddd, J = 19.4, 6.2, 5.0 Hz, H-7a and H-7′a), 2.33 (2H, ddd, J = 19.4, 9.2, 2.8 Hz, H-7b and H-7′b), 2.10 (6H, s, H-11 and H-11′), 1.59 (6H, s, H-12 and H-12′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 185.3 (C, C-9 and C-9′), 160.5 (C, C-1 and C-1′), 158.3 (C, C-4a and C-4a′), 150.8 (C, C-3 and C-3′), 135.6 (C, C-8a and C-8a′), 133.3 (CH, C-8 and C-8′), 115.9 (C, C-2 and C-2′), 109.3 (CH, C-4 and C-4′), 105.3 (C, C-9a and C-9a′), 82.3 (C, C-10a and C-10a′), 76.9 (CH, C-6 and C-6′), 75.9 (C-5 and C-5′), 57.3 (CH_3, 6-OCH_3 and 6′−OCH_3), 30.9 (CH_2, C-7 and C-7′), 21.0 (CH_3, C-11 and C-11′), 20.0 (CH_3, C-12 and C-12′); HRMS (ESI-TOF) *m/z* 579.2228 (calcd for $C_{32}H_{35}O_{10}$, 579.2230) $[M + H]^+$.

Biological Assay. Assay for activity against *P. falciparum* (K1, multidrug-resistant strain) was performed using the microculture radioisotope technique described by Desjardins.[12] IC_{50} represents the concentration that causes 50% reduction of parasite growth as indicated by the in vitro uptake of [^{3}H]-hypoxanthine by *P. falciparum*. A standard antimalarial compound, dihydroartemisinin, showed an IC_{50} value of 1.8 ng/mL in the same assay system. Cytotoxicity of the purified compounds against African green monkey kidney fibroblast (Vero), human epidermoid carcinoma cells (KB), human breast cancer cells (BC), and human lung cancer cells (NCI-H187) was evaluated using the colorimetric method.[13]

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