

## A Quinone Derivative from an Endophytic Fungus *Phomopsis* sp. from *Morus cathayana*

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**Abstract:** A new quinone derivative, named epoxyquinophomopsin (**1**), was isolated from the EtOAc extract of the fermentation broth culture of an endophytic fungus *Phomopsis* sp. from *Morus cathayana*. Structure of compound **1** was determined mainly by NMR and mass spectral data. Biogenetically compound **1** can be regarded as a degradative product of the previously reported anthraquinone found in the fungus.

**Keywords:** Quinone derivative; Quinophomopsin; *Phomopsis* sp.; *Morus cathayana*. © 2017 ACG Publications. All rights reserved.

### 1. Fungal Source

The endophytic fungus *Phomopsis* sp. was isolated November 2013 from the twigs of *Morus cathayana* growing in the garden of Natural Product Chemistry Laboratory, Institut Teknologi Bandung, Indonesia. The fungus was identified by Dr. Muhammad Ilyas, Indonesian Science Institute, Cibinong, West Java, Indonesia, using ITS4 and ITS5 rDNA regions [1,2]. This fungus was deposited at the Laboratory with a code AZ1a.

Isolation of the fungus was carried out as follows. The plant samples (twig) were sequentially sterilized with 70% EtOH, 2% sodium hypochlorite, and 70% EtOH, each for 1 minute, and then was rinsed twice with sterile waters. The sterilized samples were cut into 1 cm pieces and placed on plates of potato dextrose agar (PDA) containing streptomycin (100 mg/L). After incubating at 28°C for 5 days, the hyphal tips of the fungus transferred to PDA. The AZ1a strain was isolated and grew on PDA as a white-brown colored culture.

### 2. Previous Studies

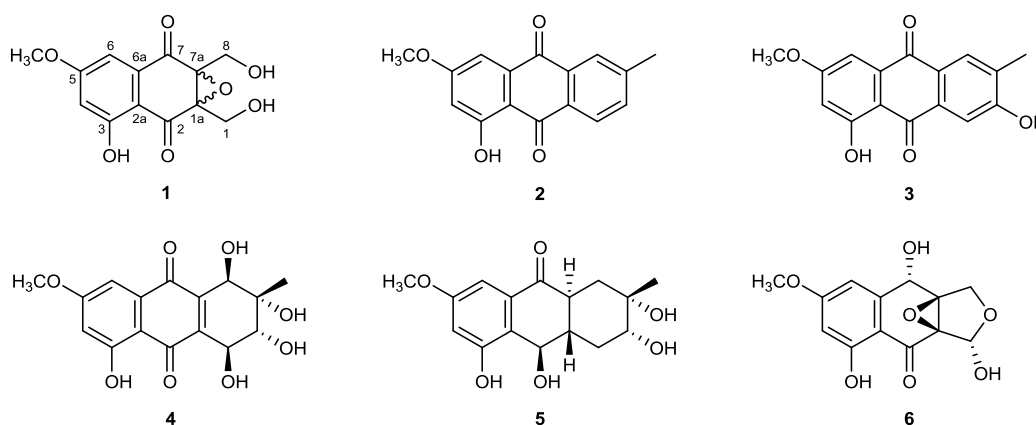
*Morus cathayana* has been described to contain the derivatives of prenylated flavonoid [3,4], 2-arylbenzofuran [5,6] and adduct Diels-Alder [7]. In the other hand, *Phomopsis* species have been reported to produce cytochalasine, terpenoid, xanthone, dimeric anthrone, lactone and anthraquinone derivatives [8].

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### 3. Present Study

*Phomopsis* sp. was cultured in 1.5 L media of potato dextrose broth (PDB). After two weeks, the mycelia were separated by filtration and the media were extracted 3 times with EtOAc (4.5 L). The combined EtOAc extract was evaporated under vacuum to give a residue (2.5 g). The residue was chromatographed with vacuum liquid chromatography (VLC) (Merck silica gel 7731, 20 g) using a stepwise elution of *n*-hexane-EtOAc (9:1-6:4) to afford seven fraction F1-F7. Fraction F2 (180 mg) was purified with centrifugal planar chromatography (CPC) (Merck silica gel 7749) eluted with CHCl<sub>3</sub>-MeOH (9:1) to give compound **1** (2 mg) as a brown gum (Figure 1).

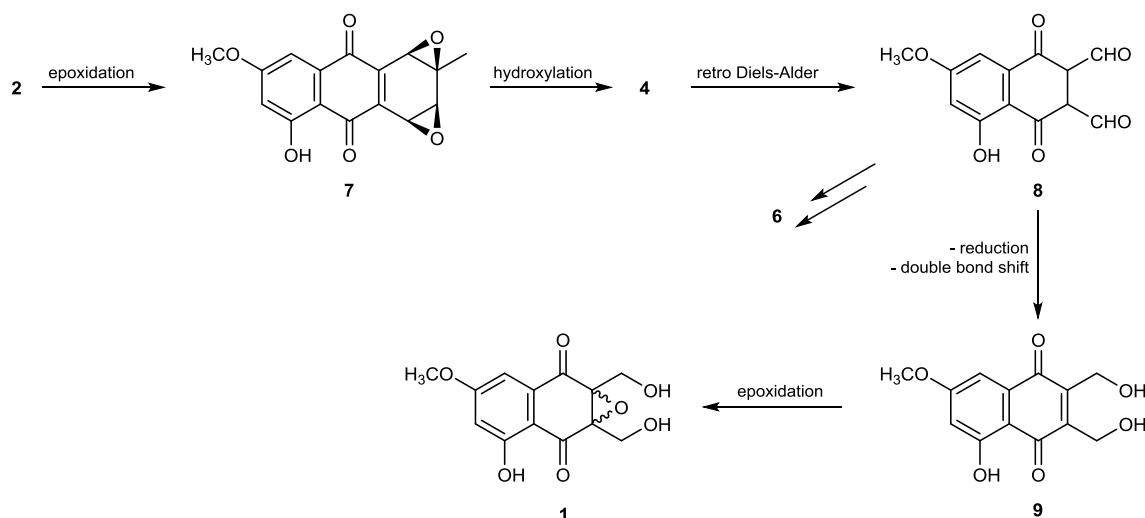
*Epoxyquinophomopsin* (**1**): Brown gum;  $[\alpha]_D^{20} = +62$  ( $c = 0.1$ , MeOH); <sup>1</sup>H NMR (Agilent DD2) (500 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = see supporting information; <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = see supporting information; HR/ESI/TOF-MS (ESI-TOF Waters Premier LCT XE):  $m/z$  [M-H]<sup>-</sup> 279.0502 (calcd. 279.0505 for [M] = C<sub>13</sub>H<sub>12</sub>O<sub>7</sub>).



**Figure 1.** Isolated anthraquinone derivatives from *Phomopsis* species

A molecular formula C<sub>13</sub>H<sub>12</sub>O<sub>7</sub> was established to compound **1** based on HRESITOFMS. The NMR spectra (<sup>1</sup>H, <sup>13</sup>C, and HSQC) of **1** showed signals for a tetrasubstituted benzene [ $\delta_C$  166.7, 164.8, 133.5, 108.9 (C), 108.1, 106.9 (CH)], two conjugated ketone ( $\delta_C$  194.2, 190.7), a tetrasubstituted epoxy ( $\delta_C$  65.6, 65.4), two primary alcohol ( $\delta_C$  58.5, 57.9;  $\delta_H$  4.85, 4.82, 3.83, 3.84), a methoxy ( $\delta_C$  56.1;  $\delta_H$  3.90), and a chelated -OH ( $\delta_H$  11.64) groups. The presence of two deshielded quaternary aromatic carbon signals ( $\delta_C$  166.7, 164.8) indicated that **1** has a partial structure of a benzoyl substituted by the -OH and methoxy group at the *ortho* and *para* positions, respectively; while the presence of a pair of *meta*-coupled aromatic proton signals ( $\delta_H$  7.11, 6.68) suggested the remaining ketone group is at the second *ortho* position in the benzoyl structure. Therefore, the remaining tetrasubstituted epoxy group should have formed a second ring with the partial structure to account for eight degree unsaturations in **1**, and consequently the two primary alcohol groups are attached to C-1a and C7a. HMBC correlations, as shown in Table 1, confirmed structure **1** for epoxyquinophomopsin. The stereochemistry of the epoxy group in **1** was not determined.

Literature search revealed that *Phomopsis* produced anthraquinone derivatives 1-hydroxy-3-methoxy-6-methylantraquinone (**2**), macrosporin (**3**), altersolanol A (**4**), and altersolanol J (**5**) [9,10] (Figure 1). However, a similar structure to those **1**, namely arthrinone (**6**), was found in the culture of *Arthrinium* sp. [11]. Structures **1-6** are biogenetically related. For example, **4** can be formed from **2** via an intermediate **7** through a series of epoxidation and hydroxylation reactions. An intermediate **9** can be produced through **8**, also by a series of reactions on **4**, including a retro Diels-Alder, a reduction and a double shift reactions. Finally, an epoxidation reaction of **9** leads to the formation of **1**. Compound **6** can also be formed by a similar reduction and a double shift reactions on the intermediate **8** (Figure 2).



**Figure 2.** Proposed biogenetic formation of **1** from **2**.

## Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

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