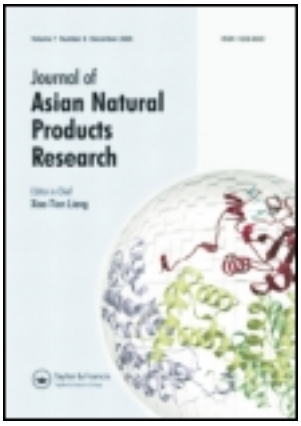

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A new phenanthrenequinone from *Dendrobium draconis*

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A new phenanthrenequinone from *Dendrobium draconis*

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A number of *Dendrobium* species (Orchidaceae) have been used as health foods. In Thailand, the tea prepared from the stems of *Dendrobium draconis* Rchb.f. (Orchidaceae) has been used as a blood tonic. Our phytochemical investigation of this plant led to the isolation of a new compound namely 5-methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone (**1**), along with four known stilbenoids including hircinol (**2**), gigantol (**3**), batatasin III (**4**), and 7-methoxy-9,10-dihydrophenanthrene-2,4,5-triol (**5**). The structures of these compounds were determined through extensive spectroscopic studies, including ^{1}H and ^{13}C NMR, DEPT, COSY, NOESY, HMQC, HMBC, ESI-MS, and HR-ESI-MS experiments. In the DPPH-free radical assay, these stilbene derivatives showed appreciable antioxidant activity.

Keywords: *Dendrobium draconis*; phenanthrenequinone; NMR; free radical scavenging

1. Introduction

Free radicals are regularly produced in living organisms as a by-product of cell metabolism. However, excessive free radicals can damage lipids, proteins, enzymes, and DNA in the cell, causing aging and several degenerative diseases [1]. Plants are rich in natural antioxidants [2], and some may be potential sources of functional foods [3]. A number of *Dendrobium* species (Orchidaceae) have been used as health foods [4,5].

Dendrobium draconis Rchb.f., locally known as 'Ueang ngoen,' is a plant growing in the northern region of Thailand, with no previous record of chemical examination. The tea prepared from the dried stems of this plant has been locally consumed for its blood tonic effect [6]. As part of our continuing studies on phenolics with antioxidative activity from Thai medicinal plants [7,8], *D. draconis* was evaluated for free radical scavenging activity. A MeOH extract obtained from the aerial parts of this plant showed 75% 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction at the concentration of 100 μg/ml. Subsequent chemical investigation of this extract resulted in the isolation of a new phenanthrenequinone namely 5-methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone (**1**), as well as four known compounds (**2**–**5**) (Figure 1). Each of these isolates was then studied for DPPH radical scavenging activity.

2. Results and discussion

Compound **1** was isolated as a reddish powder. The positive HR-ESI-MS exhibited an $[M + H]^+$ ion at *m/z* 257.0817 (calcd for 257.0813; $C_{15}H_{13}O_4$), suggesting the molecular formula of $C_{15}H_{12}O_4$. The

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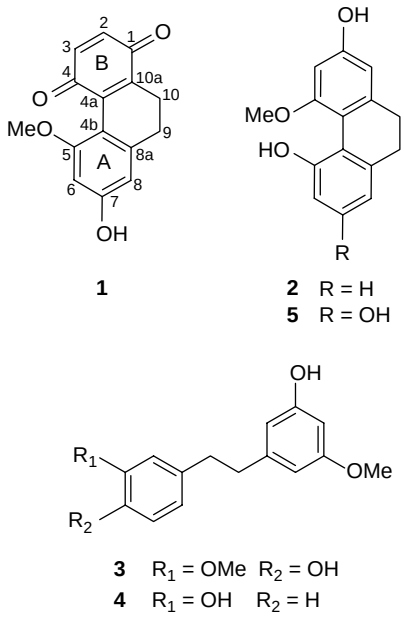

Figure 1. Structures of compounds **1**–**5**.

IR spectrum showed absorption bands for hydroxyl (3363 cm^{-1}), ketone (1733 cm^{-1}), and aromatic (1603, 1464 cm^{-1}) groups. The UV absorptions at 485 and 250 nm and the ^{13}C NMR signals at δ 185.4 (C-1) and 185.7 (C-4) were indicative of a phenanthrenequinone structure [9]. The olefinic protons at δ 6.68 (1H, d, $J = 10.0$ Hz, H-2) and 6.78 (1H, d, $J = 10.0$ Hz, H-3) exhibited HMBC correlations with C-1 and C-4, respectively (Table 1), confirming the quinone structure (ring B). Compound **1** should have a 9,10-dihydro partial structure, as suggested from the presence of ^1H NMR signals for two pairs of methylene protons at δ 2.55 (H_2-10) and 2.60 (H_2-9) [9], which correlated with the carbons at δ 20.1 (C-10) and 28.5 (C-9) in the HSQC spectrum. The ^1H NMR spectrum of **1** also showed signals for a methoxyl group (δ 3.73, s, 3H) and two *meta*-coupled aromatic protons at δ 6.31 (1H, d, $J = 2.0$ Hz) and 6.33 (1H, d, $J = 2.0$ Hz) assignable to H-8 and H-6, respectively. These assignments were confirmed by the HMBC correlation from H-8 to C-9, and the NOESY cross peak between H-8 and H_2-9 (Figure 2). From the above observations, it appears that **1** should have a structure similar to dendronone (5-hydroxy-7-methoxy-9,10-dihydrophenanthrene-1,4-dione), a phenanthrenequinone earlier reported from *Dendrobium cariniferum* and *Dendrobium longicornu* [10,11]. However, in structure **1**, the methoxyl group should be located at C-5, since the methoxyl

Table 1. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** in $CDCl_3$ (δ in ppm and J in Hz).

No.	δ_H	δ_C	HMBC (correlation with ^{13}C)
1	–	185.4 (s)	–
2	6.68 (d, $J = 10.0$)	135.1 (d)	1[a]
3	6.78 (d, $J = 10.0$)	137.2 (d)	4[a], 4a
4	–	185.7 (s)	–
4a	–	140.9 (s)	–
4b	–	112.3 (s)	–
5	–	158.9 (s)	–
6	6.33 (d, $J = 2.0$)	98.6 (d)	4b, 5[a], 7[a], 8
7	–	158.8 (s)	–
8	6.31 (d, $J = 2.0$)	107.4 (d)	4b, 6, 7[a], 9
8a	–	143.1 (s)	–
9	2.60 (m)	28.5 (t)	4b, 8, 8a[a], 10[a], 10a
10	2.55 (m)	20.1 (t)	9[a]
10a	–	139.8 (s)	–
5-OMe	3.73 (s)	55.8 (q)	5

Note: [a] Two-bond coupling.

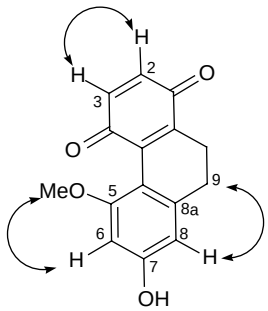

Figure 2. NOE correlations for **1**.

protons exhibited NOESY interaction with H-6, but not with H-8. This was further confirmed by the ^{2}J HMBC correlation from H-8 to C-7, which was a carbinol carbon. Based on the above spectral evidence, compound **1** was characterized as 5-methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone.

The known compounds were identified as hircinol (**2**) [12], gigantol (**3**) [13], batatasin III (**4**) [14], and 7-methoxy-9,10-dihydrophenanthrene-2,4,5-triol (**5**) [11] through comparison of their ^{1}H and ^{13}C NMR properties and MS data with those reported in the literature.

Compounds **1**–**5** are structurally related and could be considered as stilbene derivatives. Several stilbenoids are known to reduce the risk of degenerative diseases and certain cancers through their antioxidative properties [3,15,16]. In this study, **1**–**4** showed appreciable DPPH-free radical scavenging activity, with magnitude less than that of quercetin or Trolox (Table 2). Compound 7-methoxy-9,10-dihydrophenanthrene-2,4,5-triol (**5**), however, showed antioxidant potency comparable to that of Trolox (IC_{50} 10.2 and 11.7 μM, respectively). The presence of the antioxidants **1**–**5** in *D. draconis* supports the traditional use of the plant. In addition, it should be noted that the reddish color of **1** may partly contribute to the homeopathic belief on this plant.

Table 2. DPPH radical scavenging activity of compounds **1**–**5** (mean ± SD).

Compounds	Percent scavenging activity at 100 μg/ml	IC_{50} (μM)
1	54.2	283.3 ± 13.7
2	95.0	22.3 ± 1.0
3	93.2	17.7 ± 0.5
4	36.3	nd
5	85.6	10.2 ± 0.1
Quercetin	95.4	2.4 ± 0.08
Trolox®	96.4	11.7 ± 0.43

Note: nd, not determined.

3. Experimental

3.1 General experimental procedures

UV spectra were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer, and IR spectra on a Perkin-Elmer FT-IR 1760X spectrophotometer. Mass spectra were recorded on a Micromass LCT mass spectrometer (ESI-TOF-MS). NMR spectra were recorded on a Bruker Avance DPX-300 FT-NMR spectrometer or a Varian Unity INOVA-500 NMR spectrometer. Microtiter plate reading was performed on a Perkin-Elmer Victor3™ 1420 multilabel counter.

3.2 Plant material

Samples of *D. draconis* were purchased from Jatujak market, Bangkok, in September 2009, and identified by Prof. Thatree Phadungcharoen (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University). A voucher specimen (BS-092552) is deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.3 Extraction and isolation

Dried, powdered stems of *D. draconis* (1.8 kg) were extracted with MeOH (3×10 L) at room temperature to give a

viscous mass of dried extract (112 g) after evaporation of the solvent. This material was subjected to vacuum–liquid chromatography (VLC) on silica gel (*n*-hexane–EtOAc and CH_2Cl_2–MeOH gradient) to give 10 fractions (A–J). Fraction F (2.1 g) was separated by column chromatography (CC) over silica gel, eluted with *n*-hexane–EtOAc (4:1) to give seven fractions (I–VII). Fraction I (1.1 g) was separated by CC (silica gel; *n*-hexane–EtOAc, 7:3) and then further purified on Sephadex LH-20 (acetone) to furnish hircinol (**2**) (2.5 mg). Fraction II (624 mg) was subjected to Sephadex LH-20 (acetone) CC to give seven fractions. Fraction 3 (414 mg) was further subjected to repeated CC over silica gel, eluted with *n*-hexane–EtOAc (8.5:1.5) to give gigantol (**3**) (115 mg). Separation of fraction 5 (101 mg) by CC (silica gel; CH_2Cl_2–MeOH, 9.5:0.5) gave batatasin III (**4**) (48 mg). Fraction G (526 mg) was separated by CC (silica gel; *n*-hexane–EtOAc, 6:4) and then further purified on Sephadex LH-20 (acetone) to yield **1** (3 mg). Fraction H (11.7 g) was subjected to VLC on silica gel (CH_2Cl_2–MeOH gradient) to give 10 fractions. Fraction 7 (2.2 g) was separated by CC (silica gel; CH_2Cl_2–MeOH, 9:1) and then by CC (silica gel; *n*-hexane–EtOAc, 7:3) to afford 7-methoxy-9,10-dihydrophenanthrene-2,4,5-triol (**5**) (22 mg).

*3.3.1 5-Methoxy-7-hydroxy-9,10-dihydro-1,4-phenanthrenequinone (**1**)*

Reddish powder; UV λ_{max}^{MeOH} (log ε): 485 (3.5), 250 (4.0) nm; IR (film) ν_{max}: 3363, 2920, 2851, 1733, 1603, 1464, 1456, 1258 $cm^{(1}$; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectral data: see Table 1; HR-ESI-MS: *m/z* 257.0817 $[M + H]^+$ (calcd for $C_{15}H_{13}O_4$, 257.0813).

3.4 DPPH radical scavenging assay

The free radical scavenging effect of the samples was assessed by measuring their ability to decolor a methanolic solution of DPPH radical (DPPH, Sigma, St Louis, USA) as previously described [7,8]. Briefly, test samples were initially prepared as a solution in EtOH (1000 μg/ml). Each compound was first tested at the concentration of 100 μg/ml. An IC_{50} value was determined if the compound showed more than 50% inhibition. For IC_{50} analysis, twofold serial dilutions were performed to give seven concentrations. The test was done by the addition of the sample solution (20 μl) to the solution of 50 μM DPPH in EtOH (180 μl) in a 96-well microtiter plate. The reaction mixture was incubated at room temperature for 30 min, and then its absorbance at 510 nm was measured with a microplate reader. Quercetin (Sigma) and Trolox® were used as positive controls.

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