

## ***Bulbophyllum Odoratissimum*에서 추출한 새로운 페난트렌, 3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene**

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## **3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene, A New Phenanthrene from *Bulbophyllum Odoratissimum***

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**요 약.** 새로운 페난트렌 유도체인 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene는 *Bulbophyllum odoratissimum* 식물에서 추출되었고, 그 구조를 광범위한 분광학적 연구와 화학적 변형을 통해 밝혔다. 이 화합물은 인간의 백혈병 세포 K562와 HL-60, 인간의 허파선암 A549, 인간의 간암 BEL-7402, 그리고 인간의 위암 세포 SGC-7901에 대한 세포독성 테스트에서 IC<sub>50</sub>가 14.23, 10.02, 3.42, 15.36와 1.13 µg/ml로 각각 나타났다.

**주제어:** *Bulbophyllum odoratissimum*, 난초목난초과, 페난트렌, 3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene, 세포독성

**ABSTRACT.** A new phenanthrene derivative 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene was isolated from the all plant of *Bulbophyllum odoratissimum*, and its structure was elucidated by extensive spectral studies and chemical transformation. The compound displayed cytotoxicity against the growth of human leukemia cell lines K562 and HL-60, human lung adenocarcinoma A549, human hepatoma BEL-7402 and human stomach cancer cell lines SGC-7901 with IC<sub>50</sub> values of 14.23, 10.02, 3.42, 15.36 and 1.13 mg/ml respectively.

**Keywords:** *Bulbophyllum odoratissimum*, Orchidaceae, Phenanthrene, 3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene, Cytotoxicity

The genus *Bulbophyllum*, belonging to the Orchidaceae consists of about 1000 species found in Asia, America and Africa<sup>1</sup>, and contains mainly phenanthrenes and bibenzyls.<sup>2-6</sup> *B. odoratissimum* (J.E. Smith) Lindl is widely distributed in China, Nepal, Sikkim, Bhutan, India, Burma, Thailand, Laos and Vietnam and used in folk medicine to treat

tuberculosis, chronic inflammation and fracture.<sup>7</sup> Investigation on the compounds from *B. odoratissimum* have revealed the presence of phenanthrene, lignan, flavonoids, bibenzyls, phenolic glycosides, aldehydes and acids.<sup>8-10</sup> During the search for bioactive compounds from medicinal plants in Yunnan of China, we investigated *B. odoratissimum* and iso-

Table 1. NMR spectroscopic data for 1 and 1a

Position	1		1a*
	$\delta_H$	$\delta_C$	$\delta_H$
1	6.97 (s)	103.6	7.17 (s)
2	-	146.5	-
3	-	138.3	-
4	-	143.0	-
4a	-	117.4	-
4b	-	122.2	-
5	8.95 (s)	106.1	9.08 (s)
6	-	146.6	-
7	-	144.3	-
8	7.19 (s)	110.4	7.54 (s)
8a	-	126.9	-
9	7.31 (s)	122.8	7.62 (d, 8.8Hz)
10	7.31 (s)	123.7	7.56 (d, 8.8Hz)
10a	-	124.9	-
2-OCH <sub>3</sub>	3.88 (s)	54.0	3.88 (s)
4-OCH <sub>3</sub>	3.85 (s)	57.8	4.01 (s)
6-OCH <sub>3</sub>	3.97 (s)	53.8	4.12 (s)
CH <sub>3</sub> COO-	-	-	2.48 (s), 2.35 (s)

\*measured in CDCl<sub>3</sub>

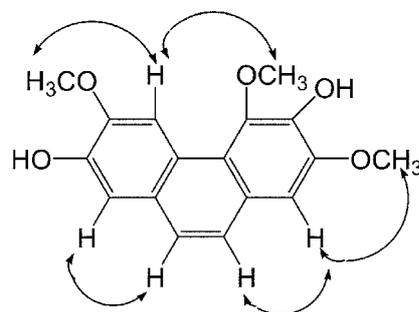
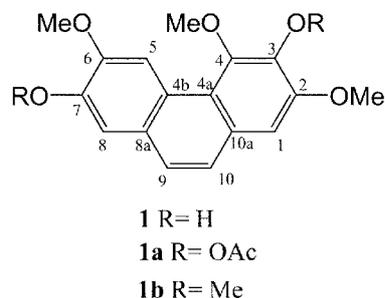


Fig. 1. ROESY correlation of 1.

lated a new phenanthrene derivative 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene (1). The isolation, structure elucidation, and evaluation for cytotoxic activity of compound 1 are described herein.

Compound **1** was obtained as a colorless amorphous powder, had the molecular formula C<sub>17</sub>H<sub>16</sub>O<sub>5</sub> by HR-ESI-MS. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra (Table 1) showed resonances for five aromatic protons [ $\delta$  8.95 (1H, s, H-5), 7.31 (2H, s, H-9,10), 7.19 (1H, s, H-8) and 6.97 (1H, s, H-1)];  $\delta$  106.1 (d, C-5), 122.8 (d, C-9), 123.7 (d, C-10), 110.4 (d, C-8) and 103.6 (d, C-1)], three methoxyl groups [ $\delta$  3.97 (3H, s, 6-OMe), 3.88 (3H, s, 2-OMe) and 3.85 (3H, s, 4-OMe)];  $\delta$  53.8 (q, 6-OMe), 54.0 (q, 2-OMe) and 57.8 (q, 4-OMe)] and nine substituted aromatic carbons. Acetylation of **1** gave a diacetate (**1a**) [ $\delta$  2.48, 2.35 (each 3H, s, CH<sub>3</sub>COO)], which displayed resonances for three isolated aromatic protons [ $\delta$  9.08 (1H, s, H-5), 7.54 (1H, s, H-8) and 7.17 (1H, s, H-1)], a pair of *ortho*-coupled aromatic protons [ $\delta_H$  7.62 and 7.56 (2H, ABq,  $J_{AB}$ =8.8 Hz, H-9 and H-10)] and three methoxyl groups [ $\delta_H$  4.12, 4.01, 3.88 (each 3H, s)], suggesting the presence of two hydroxyl groups and the absence of any substitution at C-9 or

C-10 in the parent compound. The most deshielded aromatic proton signal ( $\delta$ 8.95) was characteristic of H-5 of a phenanthrene and the absence of a second deshielded signal indicated that H-4 was substituted.<sup>3</sup> The NOE correlation in the 2D ROESY plot (Fig. 1) between H-5 and methoxyls ( $\delta$ 3.97 and 3.85) suggested that C-4 and C-6 were both linked with methoxyls, and the same observation between peak at  $\delta$ 7.31 (2H, s, H-9, 10) and signals at  $\delta$ 7.19 and 6.97 revealed the aromatic protons at C-1 and C-8. The <sup>1</sup>H signal at  $\delta$ 6.97 (H-1) correlated methoxyl signal at  $\delta$ 3.88 showed the presence of the third methoxyl group at C-2, thus the two hydroxyl groups were respectively located at C-3 and C-7. The correlations in HMBC spectrum of **1** (Fig. 2) further supported the deduction. In addition, methylation of **1** gave the known pentamethyl ether **1b**.<sup>4</sup> Finally the structure of **1** was unambiguously elucidated as 3,7-dihydroxy-2,4,6-trimethoxyphenanthrene.

Compound **1** were evaluated in vitro for its inhibitory abilities against the growth of human leukemia cell lines K562 and HL-60, human lung adenocarcinoma A549, human hepatoma BEL-7402 and human stomach cancer cell lines SGC-

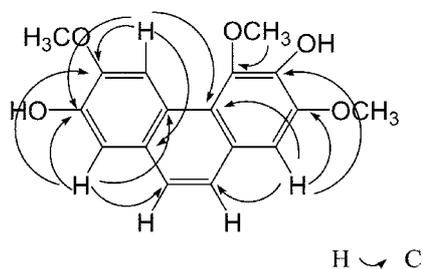


Fig. 2. HMBC correlation of **1**.

7901.<sup>11-12</sup> It displayed cytotoxicity against K562, HL-60, A549, BEL-7402 and SGC-7901 with  $IC_{50}$  values of 14.23, 10.02, 3.42, 15.36 and 1.13  $\mu\text{g/ml}$  respectively.

## EXPERIMENTAL SECTION

**General Procedures.** MS were determined on an API Qstar Pulsa LC/TOF mass spectrometer. NMR spectra were measured on a Bruker DRX-500 spectrometer with TMS as internal standard. Silica gel (200-300 mesh) was used for column chromatography and silica gel GF<sub>254</sub> for TLC (Qingdao Marine Chemical Co., China). Solvents were of industrial purity and distilled prior to use.

**Plant materials.** The whole plant of *Bulbophyllum odoratissimum* were collected from Simao County of Yunnan Province, China in February, 2004 and identified by one of the authors, Dr. Hong Wang, School of Life Science, Yunnan University, where a voucher specimen (No.0402017) was deposited.

**Extraction and isolation.** The air-dried powdered whole plant of *B. odoratissimum* (20 Kg) were extracted with 95% EtOH (20 liters  $\times$  4) at room temperature. The EtOH extract was concentrated in vacuo to yield a dark brown residue (1 Kg). H<sub>2</sub>O (2.5 L) was added to the residue, and the resultant solution was extracted with petroleum ether, EtOAc and n-BuOH successively (1.5 liters  $\times$  4). The EtOAc extract (350 g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of acetone to obtain 6 fractions. Fr. 3 (77 g) was separated to two subfractions by silica gel column chromatography (petroleum

ether-acetone 4:1, 7:3). The second subfraction (47 g) was subjected to repeated column chromatography, first on silica gel (CHCl<sub>3</sub>-acetone 80:1) and then on Sephadex LH-20 (MeOH-H<sub>2</sub>O 9:1) to obtain **1** (21 mg).

**Compound 1:** <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD) and <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD), see Table 1; EI-MS  $m/z$  (70 eV, ret. Int., %): 300 [M<sup>+</sup>] (100), 285 (63), 253 (17), 242 (22), 214 (33), 185 (11), 150 (16); HRESIMS  $m/z$ : 323.0889 [M+Na]<sup>+</sup>, requires 323.0895.

**Methylation of 1:** compound **1** (10 mg) was methylated with CH<sub>2</sub>N<sub>2</sub> in Me<sub>2</sub>CO. Preparative TLC of the crude product (silica gel, petroleum ether-EtOAc 4:1) gave 2,3,4,6,7-pentamethoxyphenanthrene (**1b**, 6 mg) as a colorless amorphous powder. EI-MS  $m/z$  (70 eV, ret. Int., %): 328 [M<sup>+</sup>] (100); <sup>1</sup>H-NMR (CD<sub>3</sub>COCD<sub>3</sub>):  $\delta$ 9.06 (1H, s, H-5), 7.61 (1H, d,  $J=9.0$  Hz, H-9), 7.57 (1H,  $J=9.0$  Hz, H-10), 7.34 (1H, s, H-1 or H-8), 7.27 (1H, s, H-8 or H-1), 4.04, 4.01, 3.99, 3.96, 3.96 (15H, each s, OMe  $\times$ 5), identical to lit. values.<sup>4</sup>

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