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

Ye-Gao Chen*, Jun-Ju Xu, Hong Yu†, Chen Qing‡,*, Yan-Li Zhang‡, Ying Liu, and Ji-Hua Wang
Department of Chemistry, Yunnan Normal University, Kunming 650092, China
†School of Life Science, Yunnan University, Kunming 650031, China
‡Yunnan Key Laboratory of Pharmacology for Natural Products Research, Kunming Medical College, Kunming 650031, China
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3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene, A New Phenanthrene from *Bulbophyllum Odoratissimum*

Ye-Gao Chen*, Jun-Ju Xu, Hong Yu†, Chen Qing‡,*, Yan-Li Zhang‡, Ying Liu, and Ji-Hua Wang
Department of Chemistry, Yunnan Normal University, Kunming 650092, China
†School of Life Science, Yunnan University, Kunming 650031, China
‡Yunnan Key Laboratory of Pharmacology for Natural Products Research, Kunming Medical College, Kunming 650031, China
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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 변형를 통해 밝혔다. 이 화합물은 인간의 백혈병 셀 K562와 HL-60, 인간의 허파선암 A549, 인간의 간암 BEL-7402, 그리고 인간의 위암셀 SGC-7901에 대한 세포독성 테스트에서 IC_{50}가14.23, 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_{50} values of 14.23, 10.02, 3.42, 15.36 and 1.13 mg/ml respectively.

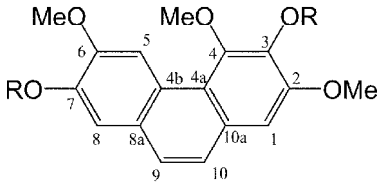
Keywords: *Bulbophyllum odoratissimum*, Orchidaceae, Phenanthrene, 3,7-Dihydroxy-2,4,6-trimethoxyphenanthrene, Cytotoxicity

The genus *Bulbophyllum*, belonging to the Orchidaceae consists of about 1000 species found in Asia, America and Africa[1], and contains mainly phenanthrenes and bibenzyls.[2-6] *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.[7] Investigation on the compounds from *B. odoratissimum* have revealed the presence of phenanthrone, lignan, flavonoids, bibenzyls, phenolic glycosides, aldehydes and acids.[8-10] 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*
	δ_H	δ_C	δ_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_3	3.88 (s)	54.0	3.88 (s)
4-OCH_3	3.85 (s)	57.8	4.01 (s)
6-OCH_3	3.97 (s)	53.8	4.12 (s)
CH_3COO-	-		2.48 (s), 2.35 (s)

*measured in $CDCl_3$



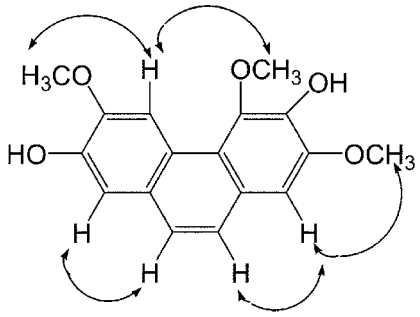


Fig. 1. ROESY correlation of 1.

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 $C_{17}H_{16}O_5$ by HR-ESI-MS. The ^{1}H-NMR and ^{13}C-NMR spectra (*Table* 1) showed resonances for five aromatic protons [δ 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); δ 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 [δ3.97 (3H, s, 6-OMe), 3.88 (3H, s, 2-OMe) and 3.85 (3H, s, 4-OMe); δ53.8 (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**) [δ2.48, 2.35 (each 3H, s, CH_3COO)], which displayed resonances for three isolated aromatic protons [δ9.08 (1H, s, H-5), 7.54 (1H, s, H-8) and 7.17 (1H, s, H-1)], a pair of *ortho*-coupled aromatic protons [δ_H 7.62 and 7.56 (2H, ABq, J_{AB}=8.8 Hz, H-9 and H-10)] and three methoxyl groups [δ_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 (δ8.95) was characteristic of H-5 of a phenanthrene and the absence of a second deshielded signal indicated that H-4 was substituted.[3] The NOE correlation in the 2D ROESY plot (*Fig.* 1) between H-5 and methoxyls (δ3.97 and 3.85) suggested that C-4 and C-6 were both linked with methoxyls, and the same observation between peak at δ7.31 (2H, s, H-9, 10) and signals at δ7.19 and 6.97 revealed the aromatic protons at C-1 and C-8. The ^{1}H signal at δ6.97 (H-1) correlated methoxyl signal at δ3.88 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**.[4] 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-

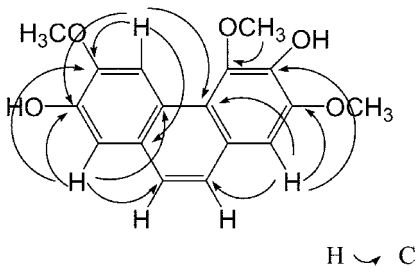


Fig. 2. HMBC correlation of **1**.

7901.[11-12] 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 μg/ml respectively.

EXPERIMENTAL SECTION

General Proceduces. 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_{254} 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 × 4) at room temperature. The EtOH extract was concentrated in vacuo to yield a dark brown residue (1 Kg). H_2O (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 ×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 ($CHCl_3$-acetone 80:1) and then on Sephadex LH-20 (MeOH-H_2O 9:1) to obtain **1** (21 mg).

Compound 1: ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD), see Table 1; EI-MS *m/z* (70 eV, ret. Int., %): 300 [M^+] (100), 285 (63), 253 (17), 242 (22), 214 (33), 185 (11), 150 (16); HRESIMS *m/z*: 323.0889 [M+Na]$^+$, requires 323.0895.

Methylation of 1: compound **1** (10 mg) was methylated with CH_2N_2 in Me_2CO. 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^+] (100); ^{1}H-NMR (CD_3COCD_3): δ9.06 (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 ×5), identical to lit. values.[4]

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