

***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 IC₅₀가 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 IC₅₀ 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

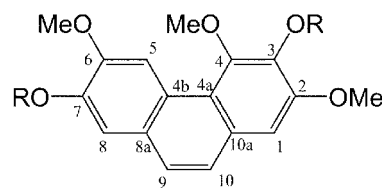
The genus *Bulbophyllum*, belonging to the Orchidaceae consists of about 1000 species found in Asia, America and Africa¹, and contains mainly phenanthrenes and bibenzyls.²⁻⁶ *B. odoratissimum* (J.E. Smith) Lindl is widely distributed in China, Nepal, Sikkim, Bhutan, India, Burma, Thailand, Laos and Vietnam 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 phenanthrene, 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 ₃ COO-	-	-	2.48 (s), 2.35 (s)

*measured in CDCl₃



1 R=H

1a R=OAc

1b R=Me

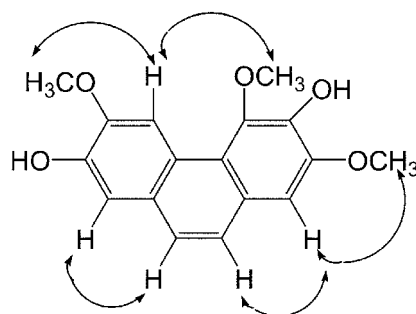


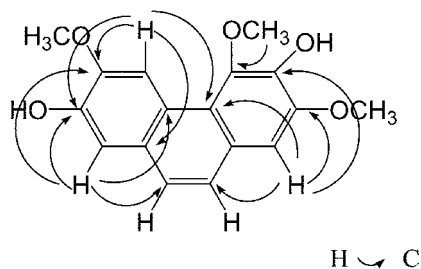
Fig. 1. ROESY correlation of **1**.

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 C₁₇H₁₆O₅ by HR-ESI-MS. The ¹H-NMR and ¹³C-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 [δ 3.97 (3H, s, 6-OMe), 3.88 (3H, s, 2-OMe) and 3.85 (3H, s, 4-OMe)]; δ 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**) [δ 2.48, 2.35 (each 3H, s, CH₃COO)], which displayed resonances for three isolated aromatic protons [δ 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 [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 (δ 8.95) 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 (δ 3.97 and 3.85) suggested that C-4 and C-6 were both linked with methoxyls, and the same observation between peak at δ 7.31 (2H, s, H-9, 10) and signals at δ 7.19 and 6.97 revealed the aromatic protons at C-1 and C-8. The ¹H signal at δ 6.97 (H-1) correlated methoxyl signal at δ 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**.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 IC₅₀ values of 14.23, 10.02, 3.42, 15.36 and 1.13 μ g/ml respectively.

EXPERIMENTAL SECTION

General Procedures. MS were determined on an API Qstar Pulsar 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 GF₂₅₄ 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). H₂O (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 (CHCl₃-acetone 80:1) and then on Sephadex LH-20 (MeOH-H₂O 9:1) to obtain **1** (21 mg).

Compound 1: ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (125 MHz, CD₃OD), 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 CH₂N₂ in Me₂CO. 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); ¹H-NMR (CD₃COCD₃): δ 8.06 (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.⁴

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REFERENCES

1. Delectis Florae Reipublicae Popularis Sinicae Agendae, Academiae Sinicae Edita. *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, **1999**, 19, 164.
2. Leong, Y. W.; Harrison, L. J. *J. Nat. Prod.* **2004**, 67, 1601-1603.
3. Leong, Y. W.; Harrison, L. J.; Powell, A. D. *Phytochemistry* **1999**, 50, 1237-1241.
4. Leong, Y. W.; Kang, C. C.; Harrison, L. J.; Powell, A. D. *Phytochemistry* **1997**, 44, 157-165.
5. Majumder, P. L.; Pal, S. *Phytochemistry* **1993**, 32, 1561-1565.
6. Majumder, P. L.; Roychowdhury, M.; Chakraborty, S. *Phytochemistry* **1997**, 44, 167-172.
7. Wu, Z. Y. *XinHuaBenCaoGangYao*. Shanghai Press of

- Science and Technology: Shanghai, 1990, Vol. 3, p 574.
8. Majumder, P. L.; Sen, R. C. *Phytochemistry* **1991**, *30*, 2092-2094.
9. Liu, D. L.; Pang, F. G.; Zhang, X.; Gao, H.; Wang, N. L.; Yao, X. S. *Acta Pharm.* **2006**, *41*, 738-741.
10. Lin, J. G.; Zhang, W. G.; Zhao, R.; Niu, Z. Y.; Bao, K.; Liu, D. L.; Wang, N. L.; Yao, X. S. *Chin. Chem. Lett.* **2006**, *17*, 307-309.
11. Mosmman, T. J. *Immunol. Meth.* **1983**, *65*, 55-63.
12. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L. *Cancer Res.* **1988**, *48*, 589-601.
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