New Bisbibenzyls from *Dendrobium falconeri*

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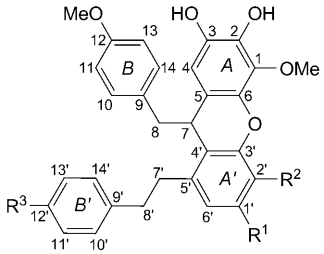
Two new bis(bibenzyls) named dendrofalconerols A and B (**1** and **2**, resp.) were isolated from the stems of *Dendrobium falconeri* (Orchidaceae), along with six known phenolic compounds which included docosanoyl (*E*)-ferulate, tetracosyl (*Z*)-*p*-coumarate, tetracosyl (*E*)-*p*-coumarate, 2-(*p*-hydroxyphenyl)ethyl *p*-coumarate, *p*-hydroxybenzoic acid, and *p*-hydroxybenzaldehyde. 2-(*p*-Hydroxyphenyl)-ethyl *p*-coumarate displayed a marginal inhibitory effect against *Herpes simplex* virus type 1, whereas the remaining compounds were devoid of antiherpetic activity.

Introduction. – The genus *Dendrobium* (Orchidaceae) is represented by more than 1,100 species widely distributed throughout Asia, Europe, and Australia, and there are about 150 species of *Dendrobium* in Thailand [1]. Plants of this genus have been known to produce a wide variety of chemical compounds, including alkaloids, bibenzyls, phenanthrenes, fluorenones, sesquiterpenes, coumarins, steroids, and polysaccharides [2]. As a part of our continuing studies on phenolics from Thai medicinal plants [3–5], we investigated the chemical constituents of the stems of *Dendrobium falconeri* HOOK., (locally known in Thai as 'Ueang Sai Wisut'), a plant growing in the northern region of Thailand with no previous record of chemical examination.

A MeOH extract prepared from the aerial parts of this plant, after repetitive chromatography, afforded two new bisbibenzyls named dendrofalconerol A (**1**) and dendrofalconerol B (**2**), along with six known phenolic compounds. These compounds were then evaluated for their inhibitory effect on the growth of *Herpes simplex* virus.



	R^1	R^2	R^3
1	MeO	OH	MeO
2	OH	H	OH

Results and Discussion. – Compound **1** was obtained as a brown amorphous powder. The HR-ESI-TOF-MS (positive ion mode) exhibited an $[M+H]^+$ ion at *m*/*z* 545.2175 (calc. for $C_{32}H_{33}O_8$: 545.2176), suggesting the molecular formula $C_{32}H_{32}O_8$. The IR spectrum showed absorption bands at 3396 (OH), 3002, 1511 (benzene ring), 1454 (CH_2), and 1245 (ether) cm^{-1}. The UV absorption at 279 nm was in agreement

with a bibenzyl structure [6]. The ^{1}H-NMR and HSQC spectra of **1** showed ten aromatic H-atoms at δ(H) 6.14–7.12. In the aliphatic region of the ^{1}H-NMR spectrum, the following H-atom signals were observed: a CH H-atom at δ(H) 4.09 (*dd*, $J = 5.5$, 7.0, H–C(7)); three pairs of CH_2 H-atoms at δ(H) 2.66–2.72, 2.76–2.82 (2*m*, CH_2(8)), 2.72–2.76, 2.86–2.90 (2*m*, CH_2(7′)), and 2.79–2.85 (*m*, CH_2(8′)); four MeO groups at δ(H) 3.70 (*s*, MeO–C(12)), 3.73 (*s*, MeO–C(12′)), 3.82 (*s*, MeO–C(1′)), and 3.89 (*s*, MeO–C(1)) (atom numbering according to [6]). The ^{13}C-NMR and DEPT spectra displayed 32 C-atom signals, corresponding to four aromatic MeO groups, three CH_2 groups, one aliphatic CH group, ten aromatic CH groups, and 14 aromatic quaternary C-atoms. Based on these spectroscopic data, the constitutional formula of **1** was proposed to be a bis(bibenzyl) structure bearing three OH and four MeO groups. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of nobilin E, a bis(bibenzyl) identified from *Dendrobium nobile* [6], revealed their structural similarity, particularly in rings *A* and *A′* with regard to the substitution patterns and the points of connection. On ring *A* of **1**, H–C(4), resonating at δ(H) 6.14 (*s*), displayed a NOESY interaction with H–C(7), and 3-bond coupling with C(2), C(6), and C(7). For ring *A′*, H–C(6′) of **1** appeared at δ(H) 6.65 (*s*). This H-atom displayed NOESY cross peaks with MeO–C(1′) (δ(H) 3.82) and H–C(7′), as well as HMBC correlations with C(2′), C(4′), and C(7′). Similar to nobilin E [6], **1** had ring *A* connected to ring *A′* through a CH bridge and an ether linkage, as shown by the HMBC correlations from H–C(7) to C(4), C(6), C(3′), and C(5′). Compound **1**, however, differed from nobilin E in the substitution pattern of the *B* and *B′* rings which were *p*-methoxylated. The first evidence came from the ^{1}H,^{1}H-COSY spectrum which showed signals for a pair of *doublet*s at δ(H) 6.61 and 6.67 (2 H each, $J = 8.5$) assignable to H–C(10/14) and H–C(11/13) of ring *B*, and another pair at δ(H) 7.12 and 6.82 (2 H each, $J = 8.5$) attributable to H–C(10′/14′) and H–C(11′/13′) of ring *B′*. This was corroborated by the NOESY cross peaks between H–C(11/13) and MeO–C(12) (δ(H) 3.70), and between H–C(11′/13′) and MeO–C(12′) (δ(H) 3.73). Further supporting information was obtained from the fragment ions at *m*/*z* 423 and 302 in the EI-MS, which were produced through two successive losses of *m*/*z* 121 from the M^+ ion. The formation of the *m*/*z* 121 ion ($[MeOC_6H_4CH_2]^+$) was due to the cleavage of the C–C bond between C(7) and C(8), or between C(7′) and C(8′), respectively. On the basis of the above spectral evidence, the structure of **1** was established as shown, and the compound was given the trivial name dendrofalconerol A. Complete ^{1}H- and ^{13}C-NMR assignments of **1** were obtained through analysis of the HMBC spectrum and are summarized in the *Table*.

Compound **2** was isolated as a brown amorphous powder. A molecular formula of $C_{30}H_{28}O_7$ was deduced from its $[M+H]^+$ ion at *m*/*z* 501.1913 (calc. for $C_{30}H_{29}O_7$: 501.1914). The UV absorption and the IR bands of **2** were similar to those of **1**, suggesting a bisbibenzyl nucleus. The first bibenzyl unit of **2** should have a structure similar to that of **1**, as indicated from the NMR data (^{1}H- and ^{13}C-NMR, HSQC, and HMBC) obtained for this moiety (*Table*). In support of this, the EI-MS of **2** showed fragment ions at *m*/*z* 121 and 379. In **2**, a CH bridge and an ether linkage were also involved in the connection between the bibenzyl units, as evident from the HMBC correlations from H–C(7) to C(6) and C(3′) of rings *A* and *A′*, respectively. On ring *A′*, a OH group was situated at C(1′), since H–C(2′) appeared as a *doublet* at δ(H) 6.38

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**. Recorded in (D_6)acetone at 500 and 125 MHz, resp.; δ in ppm, J in Hz.

	δ(H)		δ(C)		HMBC[a])	
	1	**2**	**1**	**2**	**1**	**2**
1	–	–	136.8 (*s*)	136.7 (*s*)	MeO–C(1)	MeO–C(1)
2	–	–	137.3[b]) (*s*)	137.5[b]) (*s*)	4	4
3	–	–	141.6[b]) (*s*)	141.7[b]) (*s*)	4	4
4	6.14 (*s*)	6.24 (*s*)	109.7 (*d*)	109.7 (*d*)	7	7
5	–	–	117.8 (*s*)	117.6 (*s*)	7, 8	7, 8
6	–	–	139.9 (*s*)	140.0 (*s*)	4, 7	4, 7
7	4.09 (*dd*, J = 5.5, 7.0)	4.17 (*t*, J = 6.0)	39.6 (*d*)	38.9 (*d*)	4, 8	4, 8
8	2.76–2.82 (*m*), 2.66–2.72 (*m*)	2.75–2.81 (*m*), 2.69–2.75 (*m*)	45.4 (*t*)	45.5 (*t*)	7, 10, 14	7, 10, 14
9	–	–	131.6 (*s*)	131.4 (*s*)	7, 11, 13	7, 11, 13
10	6.61 (*d*, J = 8.5)	6.55 (*d*, J = 8.5)	131.3 (*d*)	131.4 (*d*)	8, 14	8, 14
11	6.67 (*d*, J = 8.5)	6.65 (*d*, J = 8.5)	113.9 (*d*)	113.8 (*d*)	13	13
12	–	–	159.1 (*s*)	159.1 (*s*)	10, 11, 13, 14, MeO–C(12)	10, 11, 13, 14, MeO–C(12)
13	6.67 (*d*, J = 8.5)	6.65 (*d*, J = 8.5)	113.9 (*d*)	113.8 (*d*)	11	11
14	6.61 (*d*, J = 8.5)	6.55 (*d*, J = 8.5)	131.3 (*d*)	131.4 (*d*)	8, 10	8, 10
1′	–	–	147.1 (*s*)	157.2 (*s*)	6′, MeO–C(1′)	2′, 6′
2′	–	6.38 (*d*, J = 2.5)	134.0 (*s*)	101.8 (*d*)	6′	6′
3′	–	–	142.3 (*s*)	154.9 (*s*)	7	2′, 7
4′	–	–	119.1 (*s*)	115.7 (*s*)	6′, 7′, 7, 8	2′, 6′, 7, 8
5′	–	–	129.5 (*s*)	141.9 (*s*)	6′, 7′, 8′,7	7′, 8′, 7
6′	6.65 (*s*)	6.56 (*d*, J = 2.5)	108.5 (*d*)	111.9 (*d*)	7′	2′, 7′
7′	2.86–2.90 (*m*), 2.72–2.76 (*m*)	2.88–2.92 (*m*), 2.70–2.74 (*m*)	34.4 (*t*)	34.8 (*t*)	6′, 8′	6′, 8′
8′	2.79–2.85 (*m*)	2.73–2.84 (*m*)	37.5 (*t*)	37.1 (*t*)	7′, 10′, 14′	7′, 10′, 14′
9′	–	–	134.6 (*s*)	133.4 (*s*)	7′, 11′, 13′	7′, 8′, 11′, 13′
10′	7.12 (*d*, J = 8.5)	7.08 (*d*, J = 8.5)	130.2 (*d*)	130.1 (*d*)	8′, 14′	8′, 14′
11′	6.82 (*d*, J = 8.5)	6.76 (*d*, J = 8.5)	114.5 (*d*)	115.9 (*d*)	13′	13′
12′	–	–	158.9 (*s*)	156.5 (*s*)	10′, 11′, 13′, 14′, MeO–C(12′)	10′, 11′, 13′, 14′
13′	6.82 (*d*, J = 8.5)	6.76 (*d*, J = 8.5)	114.5 (*d*)	115.9 (*d*)	11′	11′
14′	7.12 (*d*, J = 8.5)	7.08 (*d*, J = 8.5)	130.2 (*d*)	130.1 (*d*)	8′, 10′	8′, 10′
*Me*O–C(1)	3.89 (*s*)	3.78 (*s*)	61.2 (*q*)	61.3 (*q*)	–	–
*Me*O–C(1′)	3.82 (*s*)	–	56.6 (*q*)	–	–	–
*Me*O–C(12)	3.70 (*s*)	3.69 (*s*)	55.3 (*q*)	55.3 (*q*)	–	–
*Me*O–C(12′)	3.73 (*s*)	–	55.4 (*q*)	–	–	–

[a]) Position of H-atoms correlating with C-atoms (optimized J(C,H) = 8 Hz). [b]) Interchangeable assignments.

(J = 2.5) due to its *m*-coupling with H–C(6′), which was assigned from its 3-bond correlation to C(7′). HMBC Connectivities were also observed from H–C(2′) to C(4′), and from C(4′) to CH_2(7). The resonances of H–C(10′/14′) at δ(H) 7.08 (2 H, *d*, J = 8.5) and H–C(11′/13′) at δ(H) 6.76 (2 H, *d*, J = 8.5) in the COSY spectrum suggested a *p*-hydroxylated *B*′ ring, and this was confirmed by the fragment ion at *m*/*z* 107

($[HOC_6H_4CH_2]^+$) in the EI-MS. Thus, it was concluded that **2** had a structure as shown, and the trivial name dendrofalconerol B was given to the compound.

So far, the only other known bis(bibenzyl) with this skeleton is nobilin E, which has been previously found in *Dendrobium nobile* [6]. Thus, the occurrence of phenolics of this type is indeed rare, and appears to be characteristic for this genus. It should be mentioned that a number of bisbibenzyls have been reported from liverworts; however, most of them contain a macrocyclic structure [7].

The other phenolics isolated from this plant were identified by comparison of their spectroscopic data with reported values. They were esters of cinnamic acid derivatives, namely docosanoyl (*E*)-ferulate [8], tetracosyl (*Z*)-*p*-coumarate [9], tetracosyl (*E*)-*p*-coumarate [10], and 2-(*p*-hydroxyphenyl)ethyl *p*-coumarate [11], as well as benzenoids including *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde [12].

All the isolated compounds were evaluated for their anti-HSV-1 activity using the plaque reduction method [3][13]. Only 2-(*p*-hydroxyphenyl)ethyl *p*-coumarate exhibited marginal activity, with an EC_{50} value of 352.1 μM against HSV-1 (acyclovir EC_{50} 0.25 μM).

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Experimental Part

General. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Milton Roy Spectronic 3000 Array* spectrophotometer. CD Spectra: *Jasco J-715* spectropolarimeter. IR Spectra: *Perkin-Elmer FT-IR 1760X* spectrophotometer. NMR Spectra: *Bruker Avance DPX-300* FT-NMR spectrometer or *Varian Unity INOVA-500* NMR spectrometer. MS: *Micromass LCT* mass spectrometer (ESI-TOF-MS) or *Thermo-Finnigan polaris Q* mass spectrometer (EI-MS).

Plant Material. The fresh stems of *D. falconeri* were purchased from Jatujak market, Bangkok, in December 2006, and identified by Prof. *Thatree Phadungcharoen* (Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University). A voucher specimen (BS-122549) is on deposit at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Extraction and Isolation. The dried stems (800 g) were powdered and extracted with MeOH (2 × 10 l, 2 d each) at r.t. The MeOH extract was filtered and evaporated under reduced pressure to give a viscous mass (73 g). This material was subjected to vacuum-liquid chromatography on SiO_2 (AcOEt/hexane gradient) to give 11 fractions, *Frs. A–K*. *Fr. D* (2.34 g) was separated by CC (SiO_2; CH_2Cl_2/hexane, gradient and AcOEt/hexane 1:4) to give 13 fractions (*Frs. I–XIII*). *Fr. IV* (99 mg) was further separated by gel filtration chromatography (*Sephadex LH20*, acetone), and then by CC (SiO_2; CH_2Cl_2/hexane 1:1) to yield docosanoyl (*E*)-ferulate (24 mg). Separation of *Fr. VIII* (154 mg) was performed on *Sephadex LH20* (acetone), and then on SiO_2 (CH_2Cl_2) to afford tetracosyl (*Z*)-*p*-coumarate (20 mg). *Fr. IX* (124 mg) was separated by CC (SiO_2; CH_2Cl_2) to give 19 fractions, *Frs. IX.1–IX.19*. Tetracosyl (*E*)-*p*-coumarate (27 mg) was obtained from *Frs. IX.2–IX.10*. *Fr. F* (1.19 g) was separated by CC (SiO_2; AcOEt/hexane, 1:4) to give 25 fractions. *Frs. F.15–F.19* (342 mg) were combined and chromatographed over *Sephadex LH20* (acetone) and then purified by CC (SiO_2; AcOEt/hexane, 1:4) to yield *p*-hydroxybenzaldehyde (10 mg). Separation of *Fr. I* (1.51 g) was performed by CC over SiO_2, eluted with AcOEt/hexane (gradient) to give 35 fractions. *p*-Hydroxybenzoic acid (110 mg) and 2-(*p*-hydroxyphenyl)ethyl *p*-coumarate (29 mg) were obtained from *Frs. I.20–I.21* and *Fr. I.27*, resp. *Fr. I.25* (216 mg) gave **1** (29 mg) and **2** (12 mg) after purification on *Sephadex LH20* (MeOH).

Dendrofalconerol A (=*4,6-Dimethoxy-9-(4-methoxybenzyl)-8-[2-(4-methoxyphenyl)ethyl]-9*H-*xanthene-2,3,5-triol*; **1**). Brown amorphous powder. $[\alpha]_D^{28} = -1.0$ ($c = 0.1$, MeOH). UV (MeOH): 279 (3.95). CD ($c = 0.05$, MeOH): 202 (+54672), 213 (+112900), 220 (−137032), 242 (−4758), 250 (−5115), 259 (−2427). IR (film): 3396, 3002, 1511, 1454, 1245. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 544 (1, M^+), 423 (100), 302 (39), 287 (15), 121 (12). HR-ESI-TOF-MS: 545.2175 ($[M+H]^+$, $C_{32}H_{33}O_8^+$; calc. 545.2176).

Dendrofalconerol B (=*8-[2-(4-Hydroxyphenyl)ethyl]-4-methoxy-9-(4-methoxybenzyl)-9*H-*xanthene-2,3,6-triol*; **2**): Brown amorphous powder. $[\alpha]_D^{28} = -3.0$ ($c = 0.1$, MeOH). UV (MeOH): 280 (3.75). CD ($c = 0.05$, MeOH): 202 (+15608), 213 (+11918), 219 (−6964), 245 (−4806), 251 (−1688), 258 (−2112). IR (film): 3392, 3005, 1511, 1457, 1245. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 500 (5, M^+), 393 (10), 379 (100), 272 (95), 121 (83), 107 (41). HR-ESI-TOF-MS: 501.1913 ($[M+H]^+$, $C_{30}H_{29}O_7^+$; calc. 501.1914).

REFERENCES

[1] G. Seidenfaden, 'Orchid genera in Thailand XII. *Dendrobium* Sw.', Opera Botanica 83, Copenhagen, 1985.
[2] G.-N. Zhang, L.-Y. Zhong, S. W. Annie Bligh, Y.-L. Guo, C.-F. Zhang, M. Zhang, Z.-T. Wang, L.-S. Xu, *Phytochemistry* **2005**, *66*, 1113.
[3] K. Likhitwitayawuid, S. Chaiwiriya, B. Sritularak, V. Lipipun, *Chem. Biodiversity* **2006**, *3*, 1138.
[4] K. Likhitwitayawuid, C. Klongsiriwet, V. Jongbunprasert, B. Sritularak, S. Wongseripipatana, *Arch. Pharm. Res.* **2006**, *29*, 199.
[5] R. Wangteeraprasert, K. Likhitwitayawuid, *Heterocycles* **2008**, *75*, 403.
[6] X. Zhang, J.-K. Xu, J. Wang, N.-L. Wang, H. Kurihara, S. Kitanaka, X.-S. Yao, *J. Nat. Prod.* **2007**, *70*, 24.
[7] J. Gorham, 'The biochemistry of the stilbenoids', Chapman & Hall, London, 1995.
[8] A. Ulubelen, G. Topcu, S. Olçal, *Phytochemistry* **1994**, *37*, 1371.
[9] L. Manfei, H. Yoshimasa, X. Guojun, N. Masatake, W. Houming, *Chin. Tradit. Herb. Drugs* **1992**, *23*, 227.
[10] J. C. del Río, A. Gutiérrez, *J. Agric. Food Chem.* **2006**, *54*, 4600.
[11] R. Kaewamatawong, N. Ruangrungsi, K. Likhitwitayawuid, *J. Nat. Med.* **2007**, *61*, 349.
[12] J. Hayashi, T. Sekine, S. Deguchi, Q. Lin, S. Horie, S. Tsuchiya, S. Yano, K. Watanabe, F. Ikegami, *Phytochemistry* **2002**, *59*, 513.
[13] K. Likhitwitayawuid, B. Supudompol, B. Sritularak, V. Lipipun, K. Rapp, R. F. Schinazi, *Pharm. Biol.* **2005**, *43*, 651.

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