Compound	Anti-viral activity EC_{50} (μM)		Cytotoxicity CC_{50} (μM)
	HSV-1	HSV-2	Vero cells
3′-*O*-Methylellagic acid-4-*O*-*β*-D-xylopyranoside (**18**)	NA	NA	nd
Artolakoochol (**19**)	NA	NA	nd
Cycloartolakoochol (**20**)	NA	NA	nd
4-Hydroxyartolakoochol (**21**)	NA	NA	nd
5,7,2′,4′-tetrahydroxy-3-prenyl-6-geranylflavone (**22**)	25.5	25.5	51.0
Acyclovir	0.25	2.85	>100

EC_{50} = Concentration that inhibits viral replication by 50%; determined only when the test compound showed > 50% inhibition at 100 μg/ml., NA = No activity (< 50% viral inhibition at 100 μg/ml)., CC_{50} = 50% inhibitory (cytotoxic) concentration against Vero cell., nd = not determined.

5. สรุปและอภิปรายผลการวิจัย

จากการศึกษาองค์ประกอบทางเคมีของเอื้องสายวิสูตร เปลือกต้นมะเกิ้มและเปลือกรากมะหาด สามารถสกัดสารบริสุทธิ์ได้ทั้งหมด 22 ชนิด เมื่อนำสารทั้งหมดมาทดสอบฤทธิ์ยับยั้งเชื้อไวรัสเริมพบว่าสาร 2-(*p*-hydroxyphenyl)ethyl *p*-coumarate สามารถยับยั้งเชื้อไวรัสเริมชนิดที่ 1 ได้เล็กน้อย สาร *β*-amyrin และ (-)-cubebin สามารถยับยั้งเชื้อไวรัสเริมทั้งชนิดที่ 1 และ 2 ได้เล็กน้อย นอกจากนี้พบว่าสาร 5,7,2′,4′-tetrahydroxy-3-prenyl-6-geranylflavone สามารถยับยั้งไวรัสเริมทั้งชนิดที่ 1 และ 2 ได้ปานกลาง

OUTPUTS

บทความวิจัยในวารสารระดับนานาชาติซึ่งอยู่ในฐานข้อมูล ISI จำนวน 3 เรื่อง

1. Sritularak, B. and Likhitwitayawuid, K. 2009. New bisbibenzyls from *Dendrobium falconeri*. Helv. Chim. Acta 92, 740-744.
2. Sritularak, B., Tantrakarnsakul, K., Likhitwitayawuid, K. and Lipipun, V. 2010. New 2-arylbenzofurans from the root bark of *Artocarpus lakoocha*. Molecules 15, 6548-6558.
3. Antiherpetic flavonoids from the root bark of *Artocarpus lakoocha*. (in preparation).

New Bisbibenzyls from *Dendrobium falconeri*

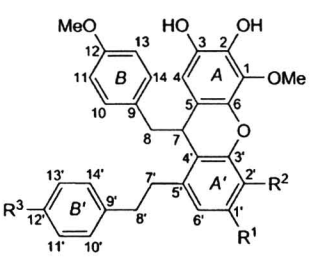
by **Boonchoo Sritularak** and **Kittisak Likhitwitayawuid***

Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand (phone: +66-2-218-8360; fax: +66-2-218-8357; e-mail: lkittisa@chula.ac.th)

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

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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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Article

New 2-Arylbenzofurans from the Root Bark of *Artocarpus lakoocha*

Boonchoo Sritularak [1], Kullasap Tantrakarnsakul [1], Kittisak Likhitwitayawuid [1,*] and Vimolmas Lipipun [2]

[1] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

[2] Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

* Author to whom correspondence should be addressed; E-Mail: Kittisak.L@chula.ac.th; Tel.: +66-221-88-360; Fax: +66-221-88-357.

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Abstract: Three new prenylated 2-arylbenzofurans – artolakoochol, 4-hydroxy-artolakoochol and cycloartolakoochol – have been isolated from the root bark of *Artocarpus lakoocha* Roxb., Their structures were elucidated through analysis of their spectroscopic data, and their antiherpetic potential was evaluated by the plaque reduction assay.

Keywords: *Artocarpus lakoocha*; Moraceae; 2-arylbenzofuran; anti-herpetic activity

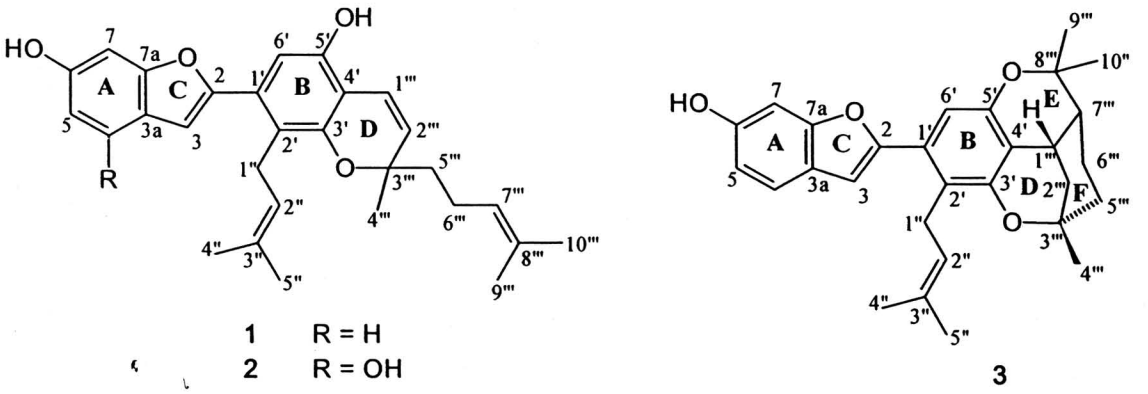
สำนักงานคณะกรรมการวิจัยแห่งชาติ ห้องสมุดงานวิจัย

1. Introduction

Artocarpus lakoocha Roxb. (Moraceae), locally known in Thai as "Ma-Haad", is a widely distributed tree in the regions of South and Southeast Asia [1]. Previous phytochemical studies of this plant have revealed the presence of triterpenoids, flavonoids and stilbenes [2-5], some of which possessed antiherpetic activity [6-8]. In an earlier report, we described the isolation of two hitherto unknown 2-arylbenzofuran-type stilbenes from the root of *A. lakoocha* [9]. In this study, a chemical investigation focusing on the root bark was undertaken, and this led to the isolation of three new prenylated 2-arylbenzofurans, namely artolakoochol (**1**), 4-hydroxyartolakoochol (**2**) and cyclo-

artolakoochol (**3**) (Figure 1). In addition, these compounds were evaluated for their inhibitory effect against *Herpes simplex* virus using the plaque reduction method.

Figure 1. Compounds isolated from the root bark of *Artocarpus lakoocha.*



2. Results and Discussion

Compound **1** was isolated as a yellow amorphous solid. The positive HR-ESI-MS exhibited an $[M+H]^+$ ion at *m/z* 445.2381 (calcd. for 445.2379; $C_{29}H_{33}O_4$), suggesting the molecular formula $C_{29}H_{32}O_4$. The IR spectrum showed absorption bands for hydroxyl (3,377 cm^{-1}), aliphatic (2,966, 2,924 and 2,854 cm^{-1}) and aromatic (1,445-1,623 cm^{-1}) groups. The UV absorptions at 234 and 339 nm were indicative of a 2-arylbenzofuran skeleton [10-11], and this was supported by the ^{1}H-NMR signal at δ 6.72 (1H, d, J = 0.5 Hz, H-3), and the ^{13}C-NMR signals at δ 105.0 (C-3) and δ 154.4 (C-2) (Table 1) [10]. The ^{13}C-NMR and HSQC spectra of **1** displayed 29 carbon signals, corresponding to five methyls, three methylenes, nine methines, and twelve quaternary carbons. The presence of a phenolic group at C-6 on ring A of the 2-arylbenzofuran nucleus was indicated by the OH signal at δ 5.29 (br s, 1H, OH-6, D_2O exchangable) and the ABM aromatic proton spin system [δ 6.75 (dd, J = 8.5, 2.0 Hz, H-5), 6.95 (d, J = 2.0 Hz, H-7) and 7.36 (d, J = 8.5 Hz, H-4) [9]. This was supported by the NOESY interactions between H-4 and H-5 (Figure 2), and further confirmed by the HMBC correlations from the OH-6 proton to C-5 (δ111.9) and C-7 (δ 98.2) (Table 1 and Figure 3).

Figure 2. Important NOESY correlations of **1** and **3**.

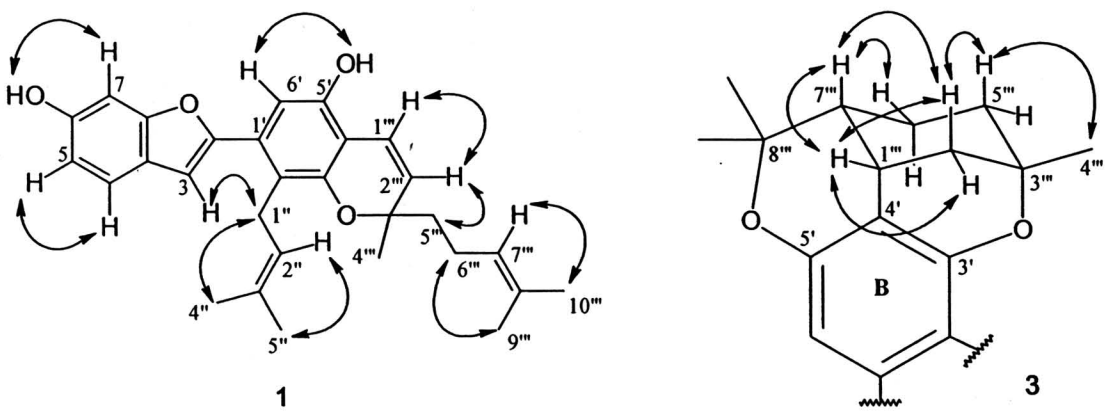
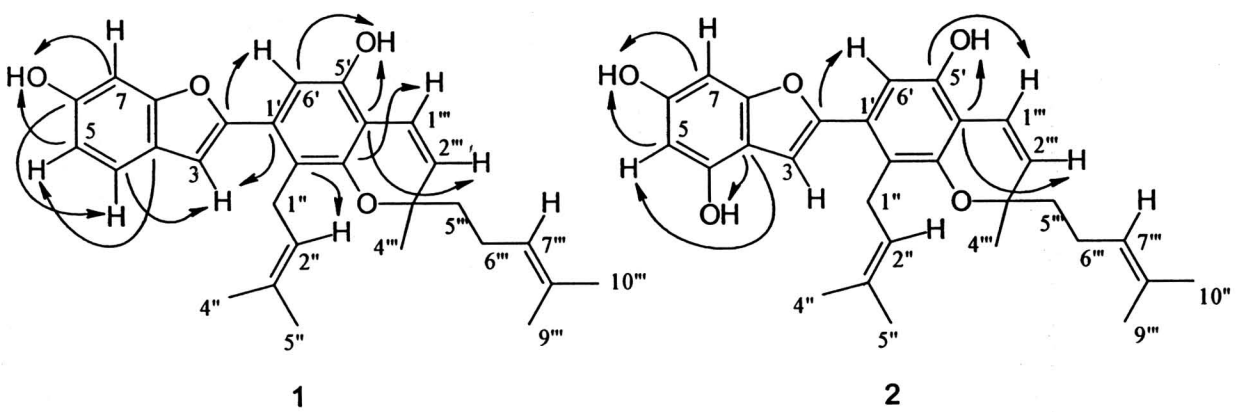


Table 1. The ^{1}H-NMR and ^{13}C-NMR data of **1** ($CDCl_3$) and **2** (acetone-d_6).

Position	δ_H		δ_C		HMBC (correlation with ^{1}H)	
	1	2	1	2	1	2
2	-	-	154.4 (s)	153.2 (s)	6′	3*, 6′
3	6.72 (d, 0.5)	6.86 (d, 1.0)	105.0 (d)	103.1 (d)	4	-
3a	-	-	122.9 (s)	112.3 (s)	3*, 5	3*, 5, 7, OH-4
4	7.36 (d, 8.5)	-	121.2 (d)	152.0 (s)	3	5*
5	6.75 (dd, 8.5, 2.0)	6.29 (d, 2.0)	111.9 (d)	98.6 (d)	7, OH-6	7, OH-6
6	-	-	153.4 (s)	157.6 (s)	4, 7*, OH-6*	5*, 7*, OH-6*
7	6.95 (d, 2.0)	6.49 (d, 2.0)	98.2 (d)	90.4 (d)	5, OH-6	5, OH-6
7a	-	-	155.2 (s)	157.7 (s)	3, 4, 7*	7*, 3
1′	-	-	130.2 (s)	131.9 (s)	3, 6′*	6′*
2′	-	-	120.1 (s)	119.0 (s)	6′, 1″*, 2″	6′, 1″*
3′	-	-	152.3 (s)	153.0 (s)	1″, 1‴	1″, 1‴
4′	-	-	109.6 (s)	110.2 (s)	6′, 1‴*, 2‴, OH-5′	6′, 1‴*, 2‴, OH-5′
5′	-	-	149.1 (s)	151.8 (s)	6′*, 1‴, OH-5′*	6′*, 1‴, OH-5′*
6′	6.70 (s)	6.80 (s)	106.9 (d)	107.6 (d)	OH-5′	-
1″	3.45 (d, 6.5)	3.47 (d, 6.5)	25.6 (t)	26.2 (t)	2″*	2″*
2″	5.17 (br t, 6.5)	5.17 (br t, 6.5)	123.6 (d)	124.8 (d)	1″*, 4″, 5″	1″*, 4″, 5″
3″	-	-	131.2 (s)	131.1 (s)	1″, 4″*, 5″*	1″, 4″*, 5″*
4″	1.72 (s)	1.74 (s)	18.1 (q)	18.3 (q)	2″, 5″	2″, 5″
5″	1.67 (s)	1.65 (s)	25.7 (q)	25.9 (q)	2″, 4″	2″, 4″
1‴	6.68 (d, 10.0)	6.74 (d, 10.5)	117.0 (d)	118.4 (d)	-	-
2‴	5.57 (d, 10.0)	5.65 (d, 10.5)	128.6 (d)	128.5 (d)	4‴, 5‴	4‴, 5‴
3‴	-	-	78.5 (s)	79.0 (s)	1‴, 2‴*, 4‴*, 5‴*, 6‴	1‴, 2‴*, 4‴*, 5‴*
4‴	1.36 (s)	1.37 (s)	26.2 (q)	26.6 (q)	2‴, 5‴	2‴
5‴	1.71 (m)	1.75 (m)	41.3 (t)	42.0 (t)	2‴, 4‴, 6‴*, 7‴	2‴, 4‴, 6‴*
6‴	2.10 (m)	2.07 (m)	22.9 (t)	23.6 (t)	5‴*, 7‴*	7‴*
7‴	5.10 (br t, 7.0)	5.13 (br t, 7.0)	124.2 (d)	125.1 (d)	5‴, 6‴*, 9‴, 10‴	5‴, 9‴, 10‴
8‴	-	-	131.7 (s)	131.4 (s)	6‴, 9‴*, 10‴*	9‴*, 10‴*
9‴	1.57 (s)	1.57 (s)	17.6 (q)	17.7 (q)	7‴, 10‴	7‴, 10‴
10‴	1.65 (s)	1.64 (s)	25.6 (q)	25.8 (q)	7‴, 9‴	7‴, 9‴
OH-4	-	8.83 (br s)		-		-
OH-6	5.29 (br s)	8.38 (br s)	-	-	-	-
OH-5′	5.19 (br s)	8.48 (br s)	-	-	-	-

*Two-bond coupling.

Figure 3. Important HMBC (C→H) correlations of **1** and **2**.



1 **2**

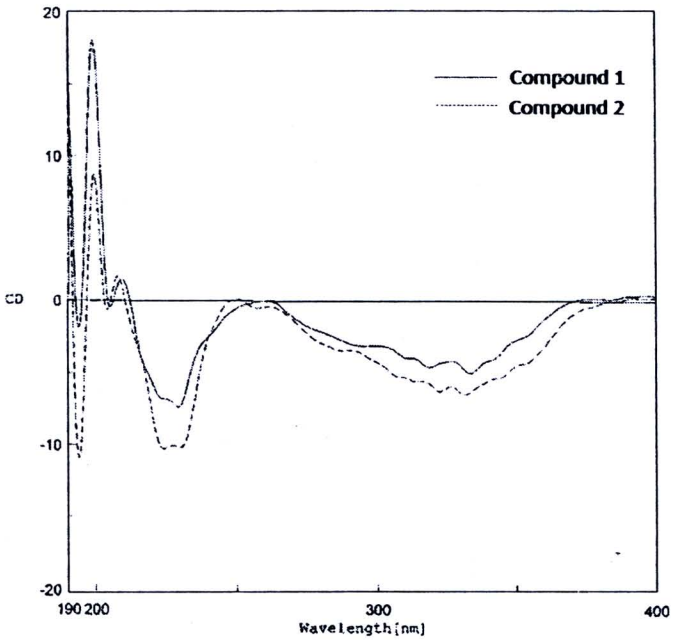
In ring B one of the *ortho*-position carbon atoms was unsubstituted, as evidenced by the HMBC correlation from C-2 to an aromatic singlet proton at δ 6.70 (H-6′). Further analysis of the ^{1}H- and ^{13}C-NMR data revealed that a 3,3-dimethylallyl group was present on the other *ortho*-position (C-2′), [^{1}H: δ 3.45 (2H, d, J = 6.5 Hz, H_2-1″), 5.17 (1H, br t, J = 6.5 Hz, H-2″), 1.67 (3H, s, H_3-5″) and 1.72 (3H, s, H_3-4″); ^{13}C: δ 25.6 (C-1″), 123.6 (C-2″), 131.2 (C-3″), 18.1 (C-4″) and 25.7 (C-5″)]. In support of this, C-2′ (δ 120.1) showed HMBC correlations to H-6′ and H-2″, and H-3 displayed NOESY interactions with H_2-1″. The ^{1}H-NMR spectrum of **1** also showed an additional phenolic proton (δ 5.19, 1H, br s, D_2O exchangeable), which could be assigned to OH-5′ from its 3-bond connectivity to C-6′ and its NOESY correlation to H-6′. Further examination of the remaining ^{1}H- and ^{13}C-NMR signals suggested that **1** also contained a modified geranyl group that formed a 2-methyl-2-(4-methylpent-3-enyl) chromene structure on ring B [^{1}H: δ 1.36 (3H, s, H_3-4‴), 1.57 (3H, s, H_3-9‴), 1.65 (3H, s, H_3-10‴), 1.71 (2H, m, H_2-5‴), 2.10 (2H, m, H_2-6‴), 5.10 (1H, br t, J = 7.0 Hz, H-7‴), 5.57 (1H, d, J = 10.0 Hz, H-2‴) and 6.68 (1H, d, J = 10.0 Hz, H-1‴); ^{13}C: δ 117.0 (C-1‴), 128.6 (C-2‴), 78.5 (C-3‴), 26.2 (C-4‴), 41.3 (C-5‴), 22.9 (C-6‴), 124.2 (C-7‴), 131.7 (C-8‴), 17.6 (C-9‴) and 25.6 (C-10‴) [12,13]. This unit should be situated at C-3′ and C-4′, and its placement was corroborated by the HMBC correlations from H-1‴ to C-3′ and C-5′, and from H-2‴ to C-4′. Based on the above spectral evidence, the structure of **1** was established as shown, and the compound was given the trivial name artolakoochol.

Compound **2**, a white powder, was analyzed for $C_{29}H_{32}O_5$ from its $[M+H]^+$ ion at *m/z* 461.2328 (calcd. for 461.2332) in the HR-ESI-MS. Its UV and IR properties were similar to those of **1**, suggesting another 2-arylbenzofuran skeleton. Comparison of the molecular formula of compound **2** with that of **1** showed that **2** should be a hydroxy derivative of **1**. This hydroxyl group should be located at C-4 of ring A, due to the absence of the H-4 resonance and the appearance of signals for H-5 and H-7, each as a doublet (J = 2.0 Hz) at δ 6.29 and δ 6.49, respectively in the ^{1}H-NMR spectrum. This was confirmed by the HMBC correlations from C-3a (δ 112.3) to OH-4 (δ 8.83, 1H, br s) and H-3 (δ 6.86, 1H, d, J = 1.0 Hz) (Table 1).

The ^{1}H- and ^{13}C-NMR signals for rings B and D of **2** resembled those of **1**. Ring B of **2** was unsubstituted at C-6′, as indicated by the three-bond coupling between H-6′ (δ 6.80, s) and C-2 (δ 153.2) in the HMBC spectrum. The presence of a 3,3-dimethylallyl group at C-2′ of ring B was supported by the HMBC correlations of C-2′ (δ 119.0) with H-6′ and H-1″ (δ 3.47, 2H, d, J = 6.5 Hz). The HMBC correlation between C-4′ (δ 110.2) and H-2‴ (δ 5.65, 1H, d, J = 10.5 Hz) confirmed the attachement of a monoterpene unit at C-4′ that was arranged to form a 2-methyl-2-(4-methylpent-3-enyl) chromene structure. Structure **2** was given the trivial name 4-hydroxyartolakoochol.

Compounds **1** and **2** were optically active with levorotation ($[\alpha]_D^{20}$ −86.1 and −117.6, respectively). Both shared similar CD properties, displaying a negative Cotton effect at 331 – 334 nm and a negative peak at 227–230 nm (Figure 4), and therefore should have the same stereochemistry at C-3‴.

Figure 4. CD data of compounds **1** and **2**.



Compound **3** was obtained as a yellow amorphous solid. A molecular formula of $C_{29}H_{32}O_4$ was deduced from its $[M+H]^+$ ion at *m/z* 445.2391 (calcd. for $C_{29}H_{33}O_4$; 445.2379). The UV absorption and IR bands of **3** were similar to those of **1**, suggesting a 2-arylbenzofuran skeleton. The benzofuran unit (rings A and C) of **3** should have a structure similar to that of **1**, as indicated from the ^{1}H-NMR signals of an ABM splitting pattern at δ 6.73 (1H, dd, J = 8.0, 2.0 Hz, H-5), δ 6.96 (1H, d, J = 2.0 Hz, H-7) and δ 7.36 (1H, d, J = 8.0 Hz, H-4), and a doublet signal at δ 6.68 (J = 1.0 Hz) assignable to proton H-3 (Table 2). The HMBC correlations of H-4 with C-3 (δ 104.3) and C-6 (δ 153.3) confirmed the presence of a phenolic group at C-6. Similar to **1** and **2**, compound **3** was unsubstituted at position 6′, as evidenced by the ^3J coupling between H-6′ (δ 6.81, 1H, s) and C-2 (δ 155.4).

The ^{1}H- and ^{13}C-NMR spectrum of **3** also exhibited signals for a 3,3-dimethylallyl group group [^{1}H: δ 3.40 (1H, dd, J = 14.5, 7.0 Hz, H-1″$_\alpha$), 3.54 (1H, dd, J = 14.5, 7.0 Hz, H-1″$_\beta$), 5.19 (1H, t, J = 7.0 Hz, H-2″), 1.66 (3H, s, H$_3$-5″) and 1.69 (3H, s, H$_3$-4″); ^{13}C: δ 25.5 (C-1″), 123.9 (C-2″), 130.9 (C-3″), 18.1 (C-4″) and 25.8 (C-5″)] which should be placed at C-2′ due to the HMBC correlations of C-2′ (δ 120.8) with H-1″. The ^{13}C-NMR, HSQC and HMBC spectra of **3** displayed, in addition to the signals for the 2-arylbenzofuran nucleus and the prenyl group, ten carbon signals corresponding to three angular methyls, three methylenes, two methines and two oxygenated quarternary carbons. This indicated that compound **3** also had a monoterpene unit which was attached to C-4′and appeared to form a tricylic structure with the oxygen functionalities on C-3′ and C-5′. The conjugation of a 10-carbon moiety to a di-*ortho* oxygenated aromatic structure to produce a pyran-cyclohexane-pyran system (rings D, E and F) has been recently observed in isorubraine, a monoterpene-chalcone conjugate isolated from the seeds of *Alpinia katsumadai* [14]. Comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of isorubraine [14] particularly on the tricyclic partial structure revealed their close similarity. Therefore the monoterpene unit should be connected to ring B by a direct linkage between

C-4′ (δ 117.2) and C-1‴ (δ 28.6) with two ether bridges between C-3′ and C-3‴, and C-5′ and C-8‴. This was supported by the HMBC correlation between H_2-2‴ (δ 1.82 and 2.21) and C-4′.

Table 2. The ^{1}H-NMR and ^{13}C-NMR data of **3** ($CDCl_3$).

Position	δ_H	δ_C	HMBC (correlation with ^{1}H)
2	-	155.4 (s)	3*, 6′
3	6.68 (d, 1.0)	104.3 (d)	4
3a	-	123.0 (s)	3*, 5, 7
4	7.36 (d, 8.0)	120.9 (d)	3
5	6.73 (dd, 8.0, 2.0)	111.6 (d)	7
6	-	153.3 (s)	4, 7*
7	6.96 (d, 2.0)	98.2 (d)	5
7a	-	155.5 (s)	3, 4, 7*
1′	-	128.4 (s)	3, 1″
2′	-	120.8 (s)	6′, 1″*
3′	-	154.9 (s)	1″
4′	-	117.2 (s)	6′, 2‴
5′	-	154.5 (s)	-
6′	6.81 (s)	109.2 (d)	-
1″ α	3.40 (dd, 14.5, 7.0)	25.5 (t)	2″*
1″ β	3.54 (dd, 14.5, 7.0)		
2″	5.19 (t, 7.0)	123.9 (d)	1″*, 4″, 5″
3″	-	130.9 (s)	1″, 4″*, 5″*
4″	1.69 (s)	18.1 (q)	2″, 5″
5″	1.66 (s)	25.8 (q)	2″, 4″
1‴	2.89 (br t, 2.0)	28.6 (d)	2‴*, 6‴
2‴ax	1.82 (dd, 13.0, 1.5)	35.1 (t)	4‴
2‴eq	2.21 (m)		
3‴	-	74.6 (s)	2‴*, 4‴*, 6‴
4‴	1.39 (s)	29.2 (q)	-
5‴ax	1.71 (m)	37.5 (t)	4‴, 6‴*
5‴eq	1.42 (m)		
6‴ax	0.70 (m)	22.3 (t)	5‴*
6‴eq	1.25 (m)		
7‴	2.05 (m)	46.9 (d)	2‴, 6‴*, 9‴, 10‴
8‴	-	83.5 (s)	6‴, 9‴*, 10‴*
9‴	1.52 (s)	29.8 (q)	10‴
10‴	1.04 (s)	23.8 (q)	9‴
OH-6	4.83 (s)	-	-

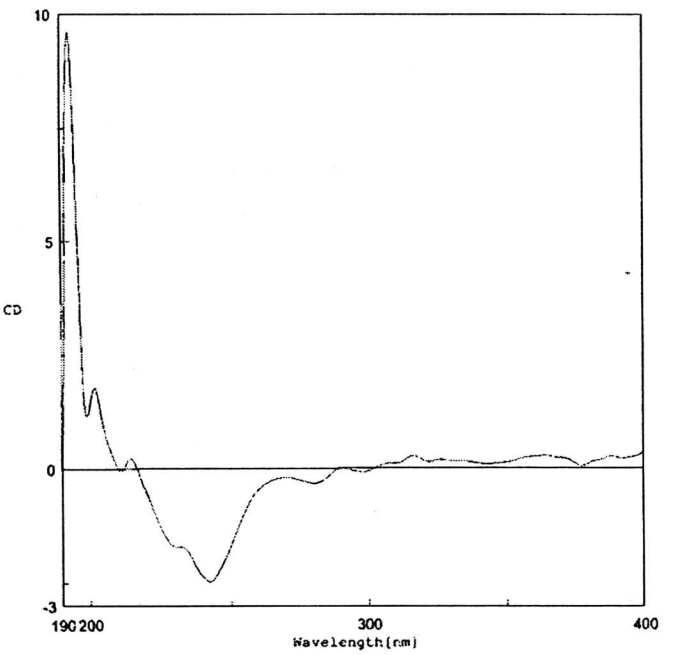
*Two-bond coupling.

The arrangement of this monoterpene unit was confirmed by HMBC correlations from C-7‴ (δ 46.9) to H_2-2‴ (δ 1.82, 1H, dd, J = 13.0, 1.5 Hz; δ 2.21, 1H, m), H_2-6‴ (δ 1.25, 1H, m; δ 0.70, 1H, m), H_3-9‴ (δ 1.52, 3H, s) and H_3-10‴ (δ 1.04, 3H, s), and from C-3‴ (δ 74.6) to H_2-2‴, H_3-4‴ (δ 1.39, 3H, s) and H_2-6‴.

The F ring of **3** appeared to have a chair conformation. Its relative configuration and NMR assignments were obtained from detailed analysis of the COSY, NOESY, HSQC and HMBC spectra. At C-2‴, the double doublet at δ 1.82 (J = 13.0, 1.5 Hz) was assigned to the axial proton from its NOESY interaction with H-7‴, whereas the multiplet at δ 2.21 was assigned to the equatorial, consistent with its long-range (W-type) coupling with equatorial H-5‴ (δ 1.42, m) observed in the COSY spectrum. The axial proton at C-5‴(δ 1.71, m), as expected, showed NOESY correlation with H_3-4‴. The equatorial proton at C-6‴ (δ 1.25, m) displayed a NOESY cross peak with H-7‴.

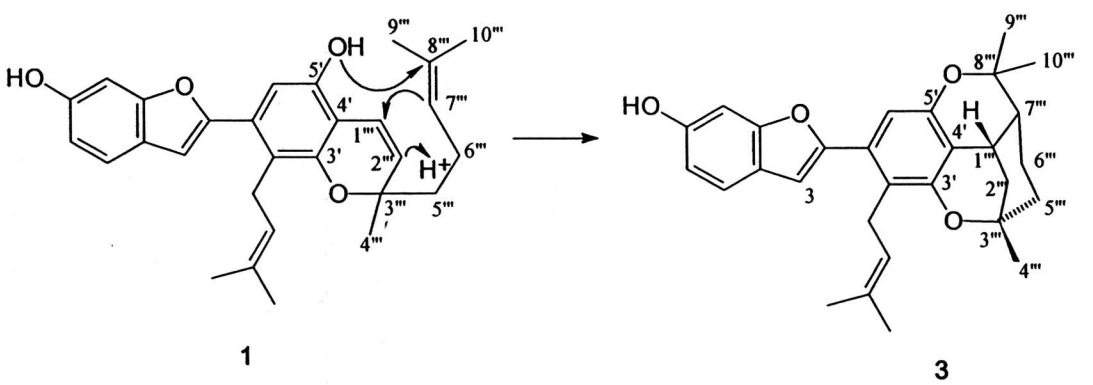
Thus, it was concluded that **3** had the structure as shown in Figure 1, and the trivial name cycloartolakoochol was given to the compound. Regarding its optical activity, **3** was found to be dextrorotatory ($[\alpha]_D^{20}$ +19.2). In the CD spectrum (Figure 5), it appeared to show a negative Cotton effect at 243 nm, although two small positive peaks at 320 and 370 nm were observed. These findings reflected the influence of the stereochemistry at C-1‴, which determined the arrangement of the tricyclic (D/E/F) ring system, on the optical properties of **3** as compared with those of **1** and **2**.

Figure 5. CD data of compound **3**.



Biogenetically, the 2-arylbenzofuran nucleus of **1** and **3** might be derived from 4,3′,5′-trihydroxystilbene (resveratrol), whereas that of **2** could be originated from 2,4,3′,5′-tetrahydroxystilbene (oxyresveratrol) [6-8,15]. Compound **3** appears to be a cyclization product of **1**.

Figure 6. Possible biogenesis of **3** from **1**.



As depicted in Figure 6, this reaction could begin with protonation of C-2‴, resulting in the formation of a carbocation at C-1‴. This would be followed by bond formation between C-1‴ and C-

7''' to give a carbocation at C-8''' that would subsequently undergo nucleophilic attack by OH-5' to produce rings E and F.

Compounds **1–3** were evaluated for their inhibitory activity against *Herpes simplex* virus types 1 and 2 (HSV-1 and HSV-2) using the plaque reduction assay [7-9], but they were devoid of activity at the concentration of 100 μg/mL.

3. Experimental

3.1. General

Optical rotations were measured on a Perkin-Elmer 341 polarimeter, and the CD spectra were recorded on a JASCO J-715 spectropolarimeter. 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. Vacuum-liquid column chromatography (VLC) and column chromatography (CC) were performed on silica gel 60 (Merck, Kieselgel 60, 70-320 mesh), silica gel 60 (Merck, Kieselgel 60, 230-400 mesh) and Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd.).

3.2. Plant Material

The root bark of *A. lakoocha* Roxb. was collected from the Botanical Garden of Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, in June 2009. Authentication was performed by comparison with herbarium specimens at the Royal Forest Department, Ministry of Agriculture and Co-operatives. A voucher specimen (BS-062552) is on deposit at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.3. Extraction and Isolation

Air dried and powdered root bark of *A. lakoocha* (2.4 kg) was successively extracted with EtOAc and MeOH (2 × 15 L, 2 days each) at room temperature, yielding an EtOAc extract (111 g) and a MeOH extract (369 g), respectively. The EtOAc extract was initially subjected to vacuum-liquid chromatography on silica gel (EtOAc-hexane gradient) to give fractions A-M. Fraction E (3.86 g) was separated by CC (silica gel; 15-20% EtOAc-hexane) to give 13 fractions. Fraction 8 (101 mg) was further separated by gel filtration chromatography (Sephadex LH-20, acetone) to give artolakoochol (**1**, 40 mg). Separation of fraction G (3.57 g) was performed on silicagel (10% EtOAc-hexane) and then on Sephadex LH-20 (acetone) to afford cycloartolakoochol (**3**, 6 mg). Fraction K (1.76 g) was separated by CC (silica gel; 30% EtOAc-hexane) to give 18 fractions. Fraction 14 (254 mg) from this column was then subjected to repeated column chromatography over silica gel (MeOH-CH_2Cl_2 1-3%)

to give a fraction which was dried and recrystallized from CH_2Cl_2 to give 4-hydroxyartolakoochol (**2**, 2.5 mg).

Artolakoochol (**1**). Yellow amorphous solid; UV (MeOH): 234 (3.16), 339 (3.23); $[\alpha]_D^{20}$ –86.1 (*c* = 0.03, MeOH); CD (MeOH, *c* 0.03): $[\theta]_{193.5}$ –3101, $[\theta]_{199}$ +30266, $[\theta]_{209}$ +2431, $[\theta]_{230}$ –12013, $[\theta]_{333.5}$ –8337 (Figure 3); IR (film) ν_{max} : 3377, 2966, 2924, 2854, 1623, 1607, 1489, 1445 cm^{-1}; HR-ESI-MS: $[M+H]^+$ at *m/z* 445.2381 (calcd. for $C_{29}H_{33}O_4$, 445.2379); ^{13}C- (125 MHz) and ^1H-NMR (500 MHz) spectral data see Table 1.

4-Hydroxyartolakoochol (**2**). White powder; UV (MeOH): 240 (3.04), 341 (3.02); $[\alpha]_D^{20}$ –117.6 (*c* = 0.03, MeOH); CD (MeOH, *c* 0.03): $[\theta]_{193.5}$ –14285, $[\theta]_{198.5}$ +11842, $[\theta]_{207}$ +2242, $[\theta]_{227}$ –14326, $[\theta]_{331}$ –9227 (Figure 3); IR (film) ν_{max} : 3417, 2965, 2920, 2855, 1633, 1609, 1447, 1418 cm^{-1}; HR-ESI-MS: $[M+H]^+$ at *m/z* 461.2328 (calcd. for $C_{29}H_{33}O_5$, 461.2332); ^{13}C- (125 MHz) and ^1H-NMR (500 MHz) spectral data see Table 1.

Cycloartolakoochol (**3**). Yellow amorphous solid; UV (MeOH): 226 (2.50), 316 (2.35); $[\alpha]_D^{20}$ +19.2 (*c* = 0.02, MeOH); CD (MeOH, *c* 0.03): $[\theta]_{194}$ +18734, $[\theta]_{199}$ +2273, $[\theta]_{202}$ +3549, $[\theta]_{214.5}$ +447, $[\theta]_{229}$ –3300, $[\theta]_{243}$ –4939 (Figure 4); IR (film) ν_{max}: 3365, 2925, 2873, 2854, 1622, 1489, 1445 cm^{-1}; HR-ESI-MS: $[M+H]^+$ at *m/z* 445.2391 (calcd. for $C_{29}H_{33}O_4$, 445.2379); ^{13}C- (125 MHz) and ^1H-NMR (500 MHz) spectral data see Table 2.

3.4. Assay of Anti-HSV Activity

Antiviral activity against HSV-1 (Strain KOS) and HSV-2 (Strain 186) was determined using the plaque reduction method, as previously described [7-9]. Briefly, virus (30 PFU/25 μL) was mixed with complete medium (25 μL) containing various concentrations of test compound and then incubated at 37 °C for 1 h. After incubation, the mixtures were added to Vero cells (6×10^5 cells/well) in 96-well microtiter plates and incubated at 37 °C for 2 h. The overlay medium containing the various concentrations of test compound was added to the Vero cells and incubated at 37 °C in humidified CO_2 incubator for 2 days. Then, virus growth inhibition was evaluated by counting the virus plaque forming on Vero cells compared with the controls. The cells also were stained with 1% crystal violet in 10% formalin for 1 h. The percent plaque inhibition was determined. Acyclovir was used as positive control.

3.5. Cytotoxicity Test

Cytotoxicity was evaluated by incubating Vero cell monolayers with completes medium containing various dilutions of sample for 72 h at 37 °C. Then, cell cytotoxicity was examined by microscopic observation [7-9].

4. Conclusions

Three new 2-arylbenzofurans: artolakoochol (**1**), 4-hydroxyartolakoochol (**2**) and cyclo-artolakoochol (**3**) were isolated from the root bark of *Artocarpus lakoocha* Roxb. All of the isolated compounds were evaluated for their anti-HSV effect, but they were devoid of activity.

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References

1. Kochummen, K.M. *The Flora of Malaya;* Ministry of Primary Industries: Kuala Lumpur, Malaysia, 1978; Volume 3, pp. 119-133.
2. Pavanasasivum, G.; Sultanbawa, M.U.S. Cycloartenyl acetate, cycloartenol and cycloartenone in the bark of *Artocarpus* species. *Phytochemistry* **1973**, *12*, 2725-2726.
3. Chauhan, J.S.; Kumari, G. A new glycoflavonol from the root bark of *Artocarpus lakoocha*. *Planta Med.* **1979**, *37*, 86-88.
4. Venkataraman, K. Wood phenolics in the chemotaxonomy of the Moraceae. *Phytochemistry* **1972**, *11*, 1571-1586.
5. Mongolsuk, S.; Robertson, A.; Towers, R. 2,4,3′,5′-Tetrahydroxystilbene from *Artocarpus lakoocha*. *J. Chem. Soc.* **1957**, 2231-2233.
6. Likhitwitayawuid, K.; Sritularak, B.; Benchanak, K.; Lipipun, V.; Mathew, J.; Schinazi, R.F. Phenolics with antiviral activity from *Millettia erythrocalyx* and *Artocarpus lakoocha*. *Nat. Prod. Res.* **2005**, *19*, 177-182.
7. Likhitwitayawuid, K.; Supudompol, B.; Sritularak, B.; Lipipun, V.; Rapp, K.; Schinazi, R.F. Phenolics with anti-HSV and anti-HIV activities from *Artocarpus gomezianus*, *Mallotus pallidus*, and *Triphasia trifolia*. *Pharm. Biol.* **2005**, *43*, 651-657.
8. Likhitwitayawuid, K.; Chaiwiriya, S.; Sritularak, B.; Lipipun, V. Antiherpetic flavones from the heartwood of *Artocarpus gomezianus*. *Chem. Biodivers.* **2006**, *3*, 1138-1143.
9. Puntumchai, A.; Kittakoop, P.; Rajviroongit, S.; Vimuttipong, S.; Likhitwitayawuid, K.; Thebtaranonth, Y. Lakoochins A and B, new antimycobacterial stilbene derivatives from *Artocarpus lakoocha*. *J. Nat. Prod.* **2004**, *67*, 485-486.
10. Yenesew, A.; Midiwo, J.O.; Guchu, S.M., Heydenreich, M.; Peter, M.G. Three isoflav-3-enes and a 2-arylbenzofuran from the root bark of *Erythrina burttii*. *Phytochemistry* **2002**, *59*, 337-341.
11. Kapche, G.D.W.F.; Fozing, C.D.; Donfack, J.H.; Fotso, G.W.; Amadou, D.; Tchana, A.N.; Bezabih, M.; Moundipa, P.F.; Ngadjui, B.T. Prenylated arylbenzofuran derivatives from *Morus mesozygia* with antioxidant activity. *Phytochemistry* **2009**, *70*, 216-221.
12. Ko, H.H.; Yu, S.M.; Ko, F.N., Teng, C.M., Lin, C.N. Bioactive constituents of *Morus australis* and *Broussonetia papyrifera*. *J. Nat. Prod.* **1997**, *60*, 1008-1011.

13. Asakawa, Y.; Kondo, K.; Tori, M.; Hashimoto, T.; Ogawa, S. Prenyl bibenzyls from the liverwort *Radula kojana*. *Phytochemistry* **1991**, *30*, 219-234.
14. Hua, S.Z., Luo, J.G.; Wang, X.B.; Wang, J.S.; Kong, L.Y. Two novel monoterpene-chalcone conjugates isolated from the seeds of *Alpinia katsumadai*. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2728-2730.
15. Reuß, S.H.; König, W.A. Corsifurans A-C, 2-arylbenzofurans of presumed stilbenoid origin from *Corsinia coriandrina* (Hepaticae). *Phytochemistry* **2004**, *65*, 3113-3118.

Sample Availability: Samples of the compounds are available from the authors.

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