Novel Biflavonoids from the Stem Bark of *Ochna integerrima*

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Two new biflavonoids, namely, 6‴-hydroxylophirone B (**1**) and 6‴-hydroxylophirone B 4‴-*O*-*β*-glucoside (**2**), were isolated from the stem bark of *Ochna integerrima*, together with five known compounds. The structures of **1** and **2** were determined by spectral data interpretation.

Ochna integerrima (Lour.) Merr. (Ochnaceae) is a tree that is widely distributed in Thailand.[1] In Thai folk medicine, the bark is used as a digestive tonic, whereas in Indonesia an infusion of the roots and leaves is used as an antidysenteric and an antipyretic.[2] Very little is known about the constituents of *O. integerrima*, and so far, there has been only one previous phytochemical investigation, which reported the isolation and structure elucidation of several new flavonoids from the leaves of this plant.[3] As part of our continuing chemical studies on Thai medicinal plants,[4] we describe herein the chromatographic separation of a MeOH extract of the stem bark of *O. integerrima*, which resulted in the isolation of two new biflavonoids, 6‴-hydroxylophirone B (**1**) and 6‴-hydroxylophirone B 4‴-*O*-*β*-glucoside (**2**), along with five known biflavonoids, 3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl)ethenyl ketone and its dihydro derivative,[5] lophirone A,[6] calodenone,[7] and lophirone C.[8]

Compound **1** was obtained as a yellow solid. The HR-FABMS showed the $[M + H]^+$ at *m/z* 527.1362 (calcd 527.1342), consistent with the molecular formula $C_{30}H_{23}O_9$. This compound showed a positive Shinoda test,[9] and its ^{13}C NMR spectum (Table 1) exhibited 30 signals, which were comprised by two carbonyls, two aliphatic methines, and 26 olefinic and aromatic carbons, suggesting a biflavonoid structure.[10] In the ^1H NMR spectrum of **1**, the typical olefinic protons of a chalcone structure at δ 7.67 and 7.72 (d, J = 15.2 Hz, each) and the characteristic aliphatic protons of a flavanone skeleton at δ 4.66 and 5.90 (d, J = 12.0 Hz, each) indicated a biflavonoid skeleton of the chalcone-flavanone type for **1**. Examination of the ^1H NMR properties of **1** in comparison with those of known biflavonoids containing a chalcone-flavanone structure revealed some similarities between **1** and lophirone B, a biflavonoid obtained from *Lophira lanceolata*,[8] except that in **1** the H-5‴ signal appeared as a doublet at δ 6.01 (J = 2.0 Hz) and the H-6‴ resonance was not evident, being replaced by a chelated phenolic proton at δ 12.27 (Table 1). In support of this, the ^{13}C NMR spectrum of **1** displayed a significant downfield shift for C-6‴ (34.7 ppm) and expected upfield shifts for C-1‴ (13.0 ppm) and C-5‴ (15.2 ppm), as compared with their counterparts in lophirone B.[8] The presence of an OH-6‴ substituent was confirmed by its long-range couplings with C-1‴ and C-5‴, and the C-3 to C-α′ interflavonoid linkage was ascertained by the correlations of H-2 to C-α′, and H-β′ to C-3 in the HMBC spectrum (Figure 1). Thus, **1** is the OH-6‴ derivative of lophirone B.[8] With regard to the stereochemistry of C-α′ and C-β′ carbons on the pyrone ring of the flavanone unit, the large vicinal coupling constant (J = 12.0 Hz) between H-α′ and H-β′ was suggestive of a *trans* relative configu-

Table 1. ^1H and ^{13}C NMR Data (Acetone-d_6) of Compounds **1** and **2**

	1		**2**	
position	^1H (J in Hz)	^{13}C	^1H (J in Hz)	^{13}C
1′		113.7		113.6
2′		166.8		166.9
3′	6.36 (1H, d, 2.3)	103.0	6.36 (1H, d, 2.3)	103.5
4′		164.8		164.7
5′	6.45 (1H, dd, 2.3, 8.9)	107.9	6.45 (1H, dd, 2.3, 9.0)	107.9
6′	8.01 (1H, d, 8.9)	132.4	8.00 (1H, d, 9.0)	132.4
CO (c)		191.9		191.8
α	7.67 (1H, d, 15.2)	117.6	7.65 (1H, d, 15.2)	117.7
β	7.72 (1H, d, 15.2)	144.2	7.71 (1H, d, 15.2)	144.1
1		126.8		126.8
2	7.55 (1H, br s)	133.4	7.55 (1H, br s)	133.4
3		123.1		122.8
4		157.9		157.8
5	6.86 (1H, d, 8.9)	115.8	6.88 (1H, d, 8.2)	115.8
6	7.56 (1H, m)	129.8	7.56 (1H, dd, 2.3, 8.2)	129.8
α′	4.66 (1H, d, 12.0)	54.3	4.70 (1H, d, 12.0)	53.5
β′	5.90 (1H, d, 12.0)	82.5	5.93 (1H, d, 12.0)	82.7
CO (c′)		197.0		197.6
1″		129.1		128.9
2″, 6″	7.29 (2H, dd, 2.0, 6.6)	129.2	7.30 (2H, dd, 2.0, 6.6)	129.2
3″, 5″	6.74 (2H, dd, 2.0, 6.6)	115.0	6.75 (2H, dd, 2.0, 6.6)	115.0
4″		157.7		157.8
1‴		102.2		102.8
2‴		163.5		163.2
3‴	6.00 (1H, d, 2.0)	95.0	6.20 (1H, d, 2.3)	95.6
4‴		166.4		166.4
5‴	6.01 (1H, d, 2.0)	96.2	6.22 (1H, d, 2.3)	97.0
6‴		164.8		165.8
1⁗			5.11 (1H, d, 7.6)	100.3
2⁗			3.47 (1H, dd, 7.6, 7.9)	73.6
3⁗			3.47 (1H, dd, 7.9, 8.9)	76.8
4⁗			3.55 (1H, dd, 8.9, 8.9)	70.2
5⁗			3.62 (1H, m)	77.1
6⁗			3.70 (1H, dd, 5.3, 11.9)	61.6
			3.88 (1H, dd, 2.6, 11.9)	
OH-2′	13.60 (1H, s)			
OH-6″	12.27 (1H, s)			

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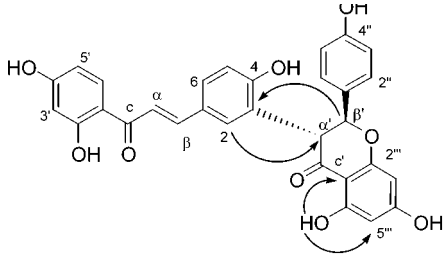


Figure 1. Key HMBC correlations for **1**.

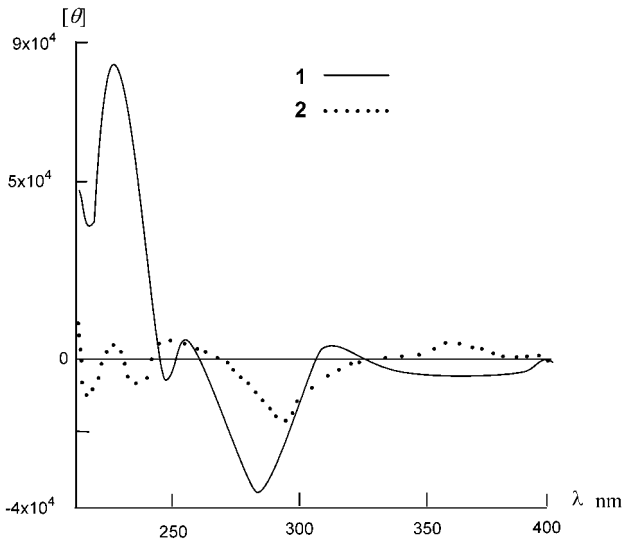
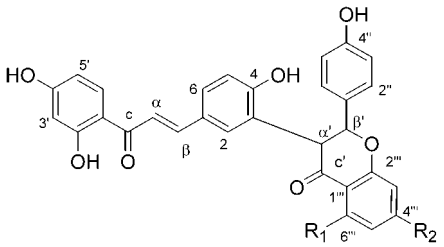


Figure 2. CD spectra of **1** and **2**.

ration. The absolute configuration was then determined by comparing the CD spectrum of **1** with that of (2*S*,3*R*)-4′,5,7-tri-*O*-methylnaringenin-(3*β*,3′)-α,2′,4,4′,6′-pentamethoxychalcone (**3**) (Figure 2). It was observed that the CD data of **1** resembled those of **3** ($[\theta]_{326.1} = +3.2 \times 10^3$, $[\theta]_{275} = -3.6 \times 10^3$),[11] exhibiting a positive Cotton effect at 323.1 nm ($[\theta] = +5.5 \times 10^3$; n → π^* transition) and a negative sign at 288.2 nm ($[\theta] = -3.7 \times 10^4$; $\pi \rightarrow \pi^*$ transition). Therefore, the absolute configuration of compound **1** was assigned α′*R*,*β*′*S*. Compound **1** [(2*S*,3*R*)-naringenin-(3*β*,3)-4,2′,4′-trihydroxychalcone] was given the trivial name 6‴-hydroxylophirone B (**1**).



	R_1	R_2
1	OH	OH
2	OH	OGlc

Compound **2** was isolated as a yellow solid. The molecular formula was determined to be $C_{36}H_{33}O_{14}$ by the $[M + H]^+$ at *m*/*z* 689.1837 (calcd 689.1870) in the HRFABMS. The ^{1}H and ^{13}C NMR spectra of **2** were reminiscent of those of **1**, with additional signals for a sugar moiety. The ^{1}H NMR signals at *δ* 5.11 (H-1‴′), 3.47 (H-2‴′), 3.47 (H-3‴′), 3.55 (H-4‴′), 3.62 (H-5‴′), 3.70, and 3.88 (H_2-6‴′), together with the coupling constant between H-1‴′ and H-2‴′ (*J* = 7.6 Hz), indicated a glucopyranosyl unit with *β*-configuration.[12] This was also corroborated by the ^{13}C NMR resonances at *δ* 100.3 (C-1‴′), 73.6 (C-2‴′), 76.8 (3‴′), 70.2 (C-4‴′), 77.1 (C-5‴′), and C-6‴′ (61.6).[12] The sugar unit was connected to the aglycon via an ether bridge linking its anomeric carbon to C-4‴ of the flavanone part, as evidenced by the NOEs of H-1‴′ with H-3‴ and H-5‴, and the HMBC coupling between H-1‴′ and C-4‴ (Figure 3). From the above spectral data, it could be concluded that **2** is 6‴-hydroxylophirone B 4‴-*O*-*β*-glucoside. Compound **2** showed positive and negative CD Cotton effects at wavelengths similar to those of **1** and **3**[11] ($[\theta]_{352.6\ nm} +3.8 \times 10^3$; n → π^* and $[\theta]_{287.0\ nm} -2 \times 10^4$; $\pi \rightarrow \pi^*$ transition), indicating that **2** also possesses the α′*R*, *β*′*S* absolute configuration.

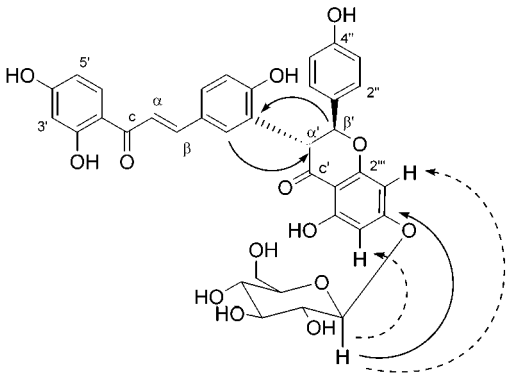


Figure 3. Selected HMBC correlations and NOE interactions for **2** → HMBC; ---> NOE.

Biflavonoids have wide distribution, being reported from bryophytes to angiosperms.[10] In the family Ochnaceae, they have been found in the genera *Brackenridgea*, *Lophira*, *Ochna*, and *Ouratea*.[3,10] It should be noted that although a number of biflavonoids have been isolated from several *Ochna* species, this is the first time that chalcone-flavanone-based flavonoid dimers have been identified from this genus.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanaco MP 53 instrument and were uncorrected. Optical rotations were measured on a JASCO DIP 140 polarimeter. UV spectra were recorded on a JASCO V 560 spectrometer. IR spectra were obtained with a JASCO FT/IR-230-IR spectrometer. CD spectra were measured on a JASCO CD J-720W spectrometer. ^{1}H NMR and NOE difference spectra were recorded on a JEOL ECP 400 or 600 spectrometer at 400 or 600 MHz, respectively, and ^{13}C NMR and HMBC ($^3J = 8$ Hz) spectra at 100 or 150 MHz on the same instruments. FABMS and HRFABMS were recorded on a JEOL JMS-HX-110A spectrometer. Thin-layer chromatography was carried out on Merck precoated Si gel (60 F_{254}) and RP_{18} ($F_{254}S$) plates. UV light was used for the detection of compounds by TLC. Column chromatography was performed using Merck Si gel (70−230 mesh). Medium-pressure liquid chromatography (MPLC) was carried out on a JASCO instrument, using Kusano CIG pre-packed columns; SiO_2 CPS-HS-221-5 (ϕ 22 × 100 mm) and ODS CPO-HS-221-20 (ϕ 22 × 100 mm). Gel filtration was performed using Sephadex LH-20 (Pharmacia).

Plant Material. The stem bark of *Ochna integerrima* (Lour.) Merr. was collected in December 1999 from Sakaeraj Environmental Reserve Station, Nakornratchasima Province, Thailand. A voucher specimen (KL122542) has been deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction and Isolation. The dried stem bark (2.5 kg) was macerated in MeOH (4 × 20 L). The MeOH extract (80 g)

was separated by Si gel column chromatography with a $CHCl_3$−MeOH gradient to give 16 fractions. Fraction 5, eluted with $CHCl_3$−MeOH (94:6), was rechromatographed on a Si gel column ($CHCl_3$−MeOH, 95:5) to furnish lophirone C (2 mg). Fraction 6, eluted with $CHCl_3$−MeOH (92:8), was separated on a Si gel column ($CHCl_3$−MeOH, 93:7) and then on a Sephadex LH-20 (MeOH) column to give 3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl)ethenyl ketone[5] (5 mg). Fraction 7, eluted with $CHCl_3$−MeOH (9:1), was further separated on Si gel ($CHCl_3$−MeOH, 93:7) and Sephadex LH-20 (MeOH) and then on an ODS MPLC (H_2O−MeOH, 35:65) column to give 3-(2,4-dihydroxybenzoyl)-2,3-dihydro-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl)ethenyl ketone[5] (5 mg). Fraction 8, eluted with $CHCl_3$−MeOH (88:12), was further separated on Si gel ($CHCl_3$−MeOH, 93:7) and Sephadex LH-20 (MeOH), and then on an ODS MPLC column (H_2O−MeOH, 30:70) to give 6‴-hydroxylophirone B (**1**, 20 mg). Fraction 9, eluted with $CHCl_3$−MeOH (86:14), was subjected to column chromatography on Si gel ($CHCl_3$−MeOH, 90:10), Sephadex LH-20 (MeOH), and ODS MPLC (H_2O−MeOH, 40:60) to give lophirone A[6] (50 mg). Further separation of fraction 10, eluted with $CHCl_3$−MeOH (84:16), on Si gel ($CHCl_3$−MeOH, 93:7) and Sephadex LH-20 (MeOH) gave calodenone[7] (6 mg). Finally, chromatographic separation of fraction 14, eluted with $CHCl_3$−MeOH (75:25), on Sephadex LH-20 (MeOH) and then by ODS MPLC (H_2O−MeOH, 50:50) yielded 6‴-hydroxylophirone B 4‴-*O*-*β*-glucoside (**2**, 2 mg).

6‴-Hydroxylophirone B (1): yellow solid (MeOH); mp 180−182 °C (uncorr); $[\alpha]^{24}_D$ −55.2° (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ϵ) 374 (5.08), 292 (4.93), 203 (5.31) nm; IR (KBr) ν_{max} 825, 1161, 1225, 1628, 3300 cm^{-1}; CD (MeOH) $[\theta]_{378.6\ nm}$ −5.4 × 10^3, $[\theta]_{323.2\ nm}$ +5.5 × 10^3, $[\theta]_{288.2\ nm}$ −3.7 × 10^4, $[\theta]_{251.8\ nm}$ +6.1 × 10^3; $[\theta]_{215.4\ nm}$ +8.6 × 10^4; ^{1}H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz), see Table 1; HRFABMS *m*/*z* $[M + H]^+$ 527.1362 (calcd for $C_{30}H_{23}O_9$ 527.1342).

6‴-Hydroxylophirone B 4‴-*O*-*β*-glucoside (2): yellow solid (MeOH); mp 263−265 °C (uncorr); $[\alpha]^{24}_D$ −0.5° (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 369 (4.30), 285 (4.30), 205 (4.70) nm; IR (KBr) ν_{max} 669, 834, 1172, 1228, 1632, 2924, 3343 cm^{-1}; CD (MeOH) $[\theta]_{352.6\ nm}$ +3.8 × 10^3, $[\theta]_{287.0\ nm}$ −2.0 × 10^4, $[\theta]_{246.6\ nm}$ +3.1 × 10^3, $[\theta]_{229.4\ nm}$ −7.5 × 10^3, $[\theta]_{217.6\ nm}$ +4.9 × 10^3, $[\theta]_{208.4\ nm}$ −1.3 × 10^4; ^{1}H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6,150 MHz), see Table 1; HRFABMS *m*/*z* $[M + H]^+$ 689.1837 (calcd for $C_{36}H_{33}O_{14}$ 689.1870).

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