

Biflavonoids from *Ochna lanceolata*

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Abstract

Two new biflavonoids, 7,4',7'',4'''-tetramethylisochamaejasmin (**1**) and 2,3-dihydroochnaflavone 7''-*O*-methyl ether (**2**), together with six known flavonoids (**3**–**8**) were isolated from the stem bark of *Ochna lanceolata*. Their structures were established on the basis of spectral studies. © 2008 Phytochemical Society of Europe. Published by Elsevier B.V. All rights reserved.

Keywords: *Ochna lanceolata*; Ochnaceae; Biflavonoids

1. Introduction

The genus *Ochna* (Ochnaceae) consists of more than 80 species distributed in tropical Asia, Africa and America (Rendle, 1952) and is found to be a rich source of biflavonoids (Anuradha et al., 2006; Ichino et al., 2006; Jayakrishna et al., 2003; Jayaprakasam et al., 2000; Kaewamatawong et al., 2002; Kamil, Khan, Alam, Ilyas, 1987; Kamil, Khan, Ilyas, & Rahman, 1983; Likhitwitayawuid, Rungserichai, Ruangrungsi, & Phadungcharoen, 2001; Messanga et al., 1998; Messanga et al., 1992; Messanga, Tih, Sondengam, Martin, & Bodo, 1994; Okigawa, Kawano, Aqil, & Rahman, 1973, 1976; Pegnyemb, Tih, Sondengam, Blond, & Bodo, 2001, 2003a, 2003b; Rao et al., 1997; Tang et al., 2003). *Ochna lanceolata* Spreng. is a semi-evergreen tree found widely in Central and Peninsular India (Matthew, 1981). The stem bark of this plant is used as an abortifacient and for treating gastric complaints and menstrual disorders (Muthukumarasamy, Mohan, & Kumarasan, 2003). As there is no record of any phytochemical work on *O. lanceolata*, we have examined the stem bark of this species and report here the isolation and characterization of two new biflavonoids, 7,4',7'',4'''-tetramethylisochamaejasmin (**1**) and 2,3-dihydroochnaflavone 7''-*O*-methyl ether (**2**), besides six known flavonoids, kaempferol (**3**), (–)-epicatechin (Khoon and Das, 1989) (**4**), afrormosin (Caballero, Smith, Fronczek, &

Fischer, 1986) (**5**), 2,3-dihydroochnaflavone (Rao et al., 1997) (**6**), ochnaflavone (Okigawa et al., 1976) (**7**) and kaempferol 3-*O*-β-D-glucopyranoside (Hari Kishore et al., 2003) (**8**).

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The presence of paired signals in the ¹³C NMR spectrum (Table 1) and [M+H]⁺ peak at *m/z* 599.1995 in the positive ESITOFMS suggested a dimeric flavonoid structure consistent with the molecular formula C₃₄H₃₀O₁₀. The UV spectrum of **1** in MeOH showed absorption maxima at 295 and 330 nm, typical of a flavanone derivative (Mabry, Markham, & Thomas, 1970a), while the IR spectrum exhibited a broad hydroxyl absorption band at 3447 cm⁻¹ and a chelated carbonyl band at 1640 cm⁻¹.

The ¹H NMR spectrum of **1** showed a two-proton D₂O exchangeable downfield signal at δ 11.90, indicating the presence of two chelated hydroxyls at the 5 and 5'' positions. The presence of two sets of *meta*-coupled doublets (*J* = 2.0 Hz) at δ 6.08 and 5.91, each integrating for two protons, were assigned to 6, 6'', and 8, 8'' protons, respectively. Two sets of *ortho*-coupled doublets (*J* = 8.0 Hz) at δ 7.22 and 7.01, integrating for four protons each, were assigned to 2', 6', 2''', 6''' and 3', 5', 3''', 5''' protons, respectively. It also showed two sets of doublets with *trans*-diaxial coupling (*J* = 12.0 Hz) at δ 5.00 (2H) and 3.92 (2H), ascribed to 2, 2'' and 3, 3'' protons, respectively of a biflavanone derivative. A sharp signal at δ 3.81, integrating for twelve protons, indicated the presence of

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Table 1
 ^{13}C NMR (75 MHz, $\text{Me}_2\text{CO}-d_6$) spectral data of **1** and **2**

Carbon	1	Carbon	Flavanone moiety of 2	Carbon	Flavone moiety of 2
2/2''	81.9	2	79.3	2''	164.4
3/3''	48.5	3	43.4	3''	105.0
4/4''	196.7	4	196.9	4''	183.0
4a/4a''	103.1	4a	102.1	4a''	105.3
5/5''	165.1	5	165.2	5''	163.3
6/6''	95.8	6	96.9	6''	99.8
7/7''	169.2	7	167.3	7''	165.0
8/8''	94.6	8	95.9	8''	94.7
8a/8a''	163.7	8a	164.1	8a''	158.8
1'/1'''	129.5	1'	133.2	1'''	125.9
2',6'/2''',6'''	130.6	2'	121.5	2'''	129.7
4'/4'''	161.6	3'	142.7	3'''	117.4
3',5'/3''',5'''	115.1	4'	150.5	4'''	162.1
7/7''-OMe	56.3	5'	118.4	5'''	117.4
4'/4'''-OMe	55.7	6'	125.5	6'''	129.7
				7''-OMe	56.0

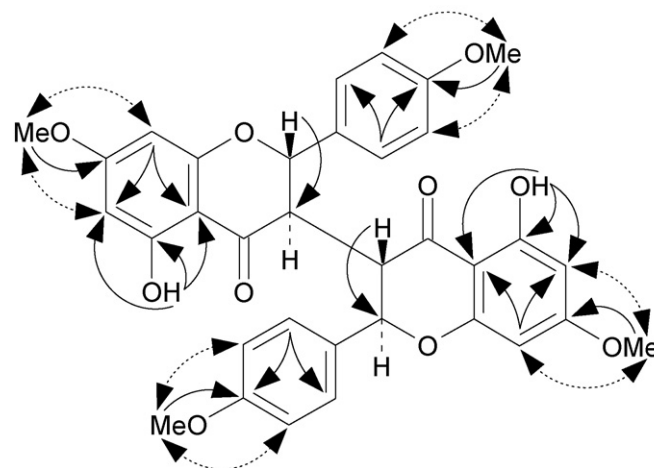
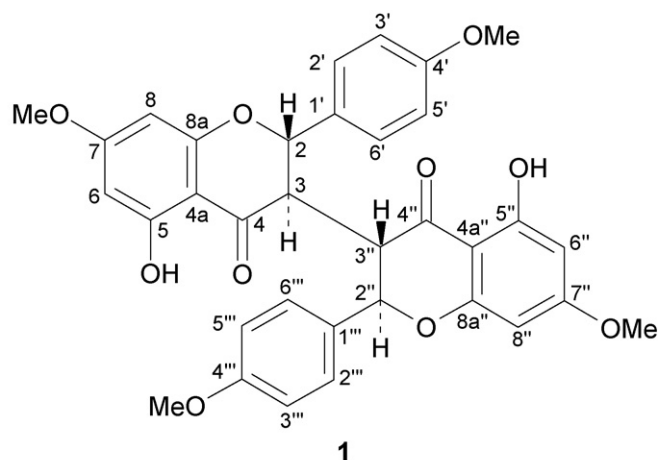


Fig. 1. Significant HMBC (—→) and NOESY (---→) correlations for **1**.

four methoxyl groups. The methoxyl groups were placed at C-7, 7'' and C-4', 4''' based on HMBC correlations, respectively, with carbons at 169.2 (C-7/7'') and 161.6 ppm (C-4'/4'''), and NOE correlations reported in Fig. 1.

Comparison of ^{13}C NMR spectral data of **1** (Table 1) with naringenin (Agrawal, 1989) showed that C-3 was involved in C–C linkage with C-3'' as the resonance of C-3 and C-3'' (48.5 ppm) was shifted downfield by 4.8 ppm from the corresponding carbon resonance of naringenin (43.7 ppm). The stereochemistry at the C-2/C-3 and C-2''/C-3'' positions were determined as *trans*–*trans* geometry by comparison of the J values (12 and 12 Hz) of the corresponding protons (H-2/H-3 and H-2''/H-3'') with the known C-3/C-3'' biflavonones (Liu, Tatematsu, Kurokawa, Niwa, & Hirata, 1984; Niwa, Chen, Liu, Tatematsu, & Hirata, 1984). Compound **1** was found to be optically inactive and attempts to determine the chirality at C-3/C-3'' positions failed, as the CD spectrum recorded for **1** did not provide any worthwhile information as in the case of other C-3/C-3'' biflavonones (Liu et al., 1984; Niwa, Jiang, & Hirata, 1986) of natural origin.

Thus, from the foregoing spectral studies, the structure of compound **1** was elucidated as 7,4',7'',4'''-tetramethylisochamaejasmin. Although compound **1** has been reported as a derivative of isochamaejasmin isolated from *Stellera chamaejasme* (Niwa et al., 1984), this is the first report of its isolation from a natural source.

Compound **2**, obtained as pale yellow needles, showed an $[\text{M}+\text{H}]^+$ peak at m/z 555.1275 in its positive ESITOFMS consistent with the molecular formula $\text{C}_{31}\text{H}_{22}\text{O}_{10}$. This was corroborated by the ^{13}C NMR spectrum, which showed signals for all the 31 carbons of the molecule. The UV spectrum showed absorption maxima at 288 and 322 nm, while the IR spectrum exhibited a broad hydroxyl absorption band at 3104 cm^{-1} and a chelated carbonyl band at 1669 cm^{-1} . The molecular formula and the presence of two carbonyl resonances at 196.9 and 183.0 ppm in its ^{13}C NMR spectrum suggested that compound **2** could be a biflavonoid.

The ^1H NMR spectrum of **2** showed an olefinic proton singlet at δ 6.64 (H-3) characteristic of a flavone nucleus, and three double doublets at δ 5.49 (H-2), 3.22 (H-3_{ax}) and 2.83 (H-3_{eq}), typical of a flavanone nucleus (Mabry, Markham, & Thomas, 1970b). Two D_2O exchangeable signals at δ 12.10 and 12.89 were assigned to chelated hydroxyls at C-5 and C-5'' positions. Two additional D_2O exchangeable signals at δ 9.58 and 8.70 were ascribed to two non-chelated hydroxyl groups in **2**. Four protons comprising two sets of *meta*-coupled doublets at δ 6.01 and 6.04 ($J = 2.4$ Hz), and 6.25 and 6.49 ($J = 2.2$ Hz), were assigned to 6 and 8 protons of ring A of flavanone moiety; and 6'' and 8'' protons of ring D of flavone moiety, respectively. This implies that these carbons are not involved in interflavonoid linkage. The signals at δ 7.09 (1H, d, $J = 8.8$ Hz), 7.30 (1H, d, $J = 2.2$ Hz) and 7.31 (1H, dd, $J = 8.8, 2.2$ Hz) correspond to 5', 2', and 6' protons of ring B of the flavanone moiety. A set of *ortho*-coupled doublets ($J = 9.0$ Hz) at δ 8.01 and 7.12, each integrating for two protons, were assigned to 2''', 6''' and 3''', 5''' protons, respectively, of a *para*-substituted aromatic ring E of flavone moiety. The remaining signal at δ 3.87, integrating for three protons

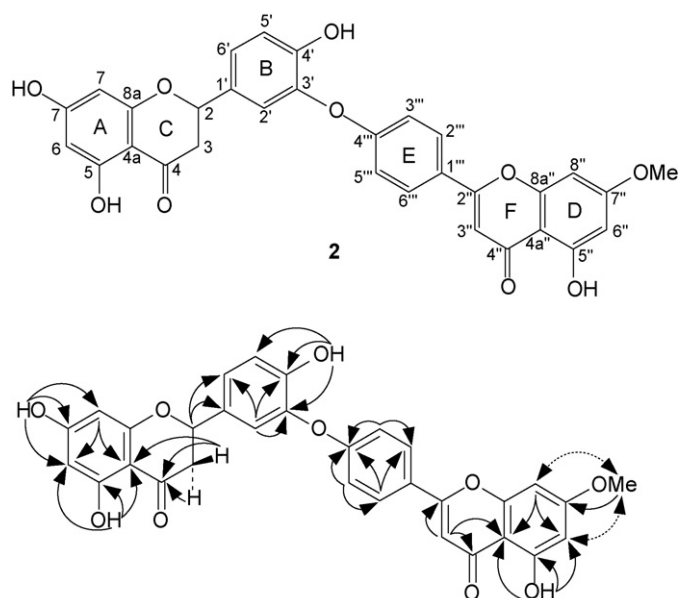


Fig. 2. Significant HMBC (—→) and NOESY (---→) correlations for **2**.

indicated the presence of a methoxyl group and was placed at C-7'' as it showed 3J correlation with this carbon at 165.0 ppm in its HMBC spectrum and NOE correlations with H-6'' (δ 6.25) and H-8'' (δ 6.49) in its NOESY spectrum (Fig. 2). From ^1H – ^{13}C long-range correlations, the two non-chelated hydroxyls at δ 9.58 and 8.70 were located at C-7 and C-4' positions of ring A and B, respectively. The foregoing spectral observations accounted for nine out of ten oxygen atoms in **2**. This suggested that the remaining oxygen atom in **2** was involved in interflavonoid ether linkage between the flavone and flavanone moiety.

Comparison of ^{13}C NMR spectral data of **2** (Table 1) with naringenin (Agrawal, 1989) and 7-*O*-methylchrysin (Harborne and Mabry, 1982) revealed that the resonances of C-3' of ring B and C-4'' of ring E in **2** have shifted downfield by 26.5 and 30.0 ppm, respectively showing that these two carbons are involved in interflavonoid ether linkage (Markham et al., 1987). The HMBC spectrum of **2** further confirmed the involvement of C-3' and C-4'' in the interflavonoid ether linkage, as these carbons showed cross peaks with H-2'/H-5' and OH-4', and H-2'''/H-6''', and H-3'''/H-5''', respectively. The absolute configuration at C-2 was shown to be *S* (Gaffield, 1970) as the CD spectrum of **2** exhibited positive and negative Cotton effects at 334 and 288 nm, respectively. Thus, from the foregoing spectral studies, compound **2** was characterized as 2,3-dihydrochonaflavone 7''-*O*-methyl ether.

3. Experimental

3.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured in CHCl_3 and MeOH at 25 °C on a PerkinElmer 241 polarimeter. UV spectra were obtained in MeOH on a

Shimadzu UV-550 spectrophotometer and IR spectra were recorded in KBr discs on a PerkinElmer 283 double beam spectrophotometer. The CD spectrum was recorded in MeOH at 25 °C on a JASCO J 715 spectropolarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer or Bruker AM 300.13 spectrometers operating at 300 and 75 MHz using $\text{Me}_2\text{CO}-d_6$ with TMS as internal standard. HMBC and NOESY (500 ms mixing time) spectra were obtained using the standard pulse sequences. ESITOFMS was recorded in positive mode on a API Q-STAR PULSA of Applied Bio-system. Column chromatography was performed on silica gel (Acme) finer than 200 mesh (0.08 mm).

3.2. Plant material

The stem bark of *O. lanceolata* was collected from the natural forest of Thanniparai, Grizzled Giant Squirrel Wildlife Sanctuary, Western Ghats, Srivilliputhur, Tamil Nadu, India, in January 2005. A voucher specimen (DG-009) documenting its collection is on deposit at the Department of Botany, Sri Venkateswara University, Tirupati, India.

3.3. Extraction and isolation

The shade dried and powdered stem bark (200 g) of *O. lanceolata* was extracted successively with *n*-hexane (3 × 1.5 L), Me_2CO (3 × 1.5 L) and MeOH (3 × 1.5 L) at room temperature. The *n*-hexane extract on purification over a Si gel column using hexane/EtOAc step gradients (9:1, 7:3 and 3:7) afforded **1** (9 mg), **3** (10 mg) and **4** (6 mg). The Me_2CO extract on purification over a Si gel column using hexane/EtOAc (8:2, 6:4, 4:6 and 3:7), and EtOAc step gradients to yield **2** (7 mg), **5** (6 mg), **6** (15 mg), **7** (13 mg) and **8** (14 mg), respectively.

3.4. 7,4',7'',4'''-Tetramethylisochamaejasmin (**1**)

White amorphous powder (MeOH); mp 262–264 °C; $[\alpha]_{\text{D}}^{28}$ 0° (*c* 0.4, MeOH); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 295 (4.21), 330 (3.47); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3447 (–OH), 2925 (–OMe), 2849, 2345, 1742, 1640 ($\text{C}=\text{O}$), 1575, 1513, 1445, 1383, 1346, 1306, 1286, 1254, 1204, 1160, 1091, 1032, 1051, 885, 834, 802, 780, 740; ^1H NMR (400 MHz, $\text{Me}_2\text{CO}-d_6$): δ 11.90 (2H, s, OH-5, 5''), 7.22 (4H, d, J = 8.0 Hz, H-2', 6', 2'', 6''), 7.01 (4H, d, J = 8.0 Hz, H-3', 5', 3'', 5''), 6.08 (2H, d, J = 2.0 Hz, H-6, 6''), 5.91 (2H, d, J = 2.0 Hz, H-8, 8''), 5.00 (2H, d, J = 12.0 Hz, H-2, 2''), 3.92 (2H, d, J = 12.0 Hz, H-3, 3''), 3.81 (12H, s, OMe-7, 4', 7'', 4''); ^{13}C NMR: see Table 1; ESITOFMS (positive mode) m/z (rel. int.): 599.1995 $[\text{M}+\text{H}]^+$ (100) (calc. for $\text{C}_{34}\text{H}_{31}\text{O}_{10}$ 599.1917).

3.5. 2,3-Dihydrochonaflavone 7''-*O*-methyl ether (**2**)

Pale yellow needles (MeOH); mp 247–248 °C; $[\alpha]_{\text{D}}^{28}$ –26.0° (*c* 0.30, CHCl_3); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 288 (4.78), 322 (4.70); +NaOAc: 284, 324; +NaOAc/ H_3BO_3 : 284, 324; + AlCl_3 : 305, 384; + AlCl_3/HCl : 305, 384; CD (MeOH, *c* 0.05): $[\theta]_{288} -7998^\circ$, $[\theta]_{334} +2365^\circ$; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3104 (–OH), 1669

($\text{C}=\text{O}$), 1630, 1584, 1485, 1446, 1369, 1300, 1154, 1098, 1085, 1023, 830; ^1H NMR (300.13 MHz, $\text{Me}_2\text{CO}-d_6$): δ 12.89 (1H, s, OH-5''), 12.10 (1H, s, OH-5), 9.58 (1H, s, OH-7), 8.70 (1H, s, OH-4') (all -OH groups exchangeable with D_2O), 8.01 (2H, d, $J = 9.0$ Hz, H-2''', 6'''), 7.31 (1H, dd, $J = 8.8, 2.2$ Hz, H-6'), 7.30 (1H, d, $J = 2.2$ Hz, H-2'), 7.12 (2H, d, $J = 9.0$ Hz, H-3''', 5'''), 7.09 (1H, d, $J = 8.8$ Hz, H-5'), 6.64 (1H, s, H-3''), 6.49 (1H, d, $J = 2.2$ Hz, H-8''), 6.25 (1H, d, $J = 2.2$ Hz, H-6''), 6.04 (1H, d, $J = 2.4$ Hz, H-8), 6.01 (1H, d, $J = 2.4$ Hz, H-6), 5.49 (1H, dd, $J = 12.7, 3.0$ Hz, H-2), 3.87 (3H, s, OMe-7''), 3.22 (1H, dd, $J = 17.1, 12.7$ Hz, H-3_{ax}), 2.83 (1H, dd, $J = 17.1, 3.0$ Hz, H-3_{eq}); ^{13}C NMR: see Table 1; ESITOFMS (positive mode) m/z (rel. int.): 555.1275 $[\text{M}+\text{H}]^+$ (100) (calc. for $\text{C}_{31}\text{H}_{23}\text{O}_{10}$ 555.1291).

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