7-*O*-Methyltetrahydroochnaflavone, a New Biflavanone from *Ochna beddomei*

B. Jayaprakasam,[†] A. G. Damu,[†] K. V. Rao,[‡] D. Gunasekar,*[,†] A. Blond,[§] and B. Bodo[§]

Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, India, Dr. Reddy's Research Foundation, Hyderabad 500 050, India, and Laboratoire de Chimie des Substances Naturelles, URA 401 CNRS, Museum National d'Histoire Naturelle, 63 Rue Buffon, 75005 Paris, France

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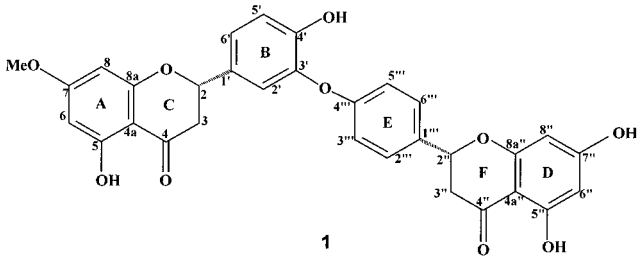
7-*O*-Methyltetrahydroochnaflavone (**1**), a new biflavanone, together with nine known flavonoids, afrormosin (**2**), 2,3-dihydroochnaflavone 7-*O*-methyl ether (**3**), kaempferol (**4**), 2,3-dihydroochnaflavone (**5**), ochnaflavone (**6**), (−)-epicatechin (**7**), kaempferol 3-*O*-rhamnoside (**8**), taxifolin 3-*O*-rhamnoside (**9**), and kaempferol 3-*O*-glucoside (**10**), were isolated from the leaves of *Ochna beddomei*, and the structures were elucidated by spectral and chemical studies.

The genus *Ochna* (Ochnaceae) is rich in biflavonoids.[1−8] *Ochna beddomei* Gamble is a deciduous tree found widely in Central and Peninsular India.[9] In continuation of our phytochemical studies on the genus *Ochna*,[8,10,11] we investigated the leaves of *O. beddomei*, a plant hitherto not examined for its chemical constituents, and report here the isolation and characterization of a new biflavanone, 7-*O*-methyltetrahydroochnaflavone (**1**) along with nine known flavonoids (**2**−**10**). The structures of the known compounds were elucidated by comparison of their physical and spectral data with the published data.



1

7-*O*-Methyltetrahydroochnaflavone (**1**) was obtained as colorless needles. The EIMS of **1** showed a molecular ion at *m*/*z* 556, and the HRFABMS of **1** exhibited the protonated molecular ion at *m*/*z* 557.1453 consistent with the molecular formula $C_{31}H_{24}O_{10}$. It gave a deep violet ferric reaction and an orange-red color with $NaBH_4$/HCl characteristic of a flavanone. The UV spectrum of **1** in MeOH exhibited absorption maxima at 285 and 330 nm, typical of a naringenin derivative.[12]

The ^{1}H NMR spectrum of **1** showed two sets of ABX signals at δ 2.81 (1H, dd, J = 17.2, 3.0 Hz), 3.20 (1H, dd, J = 17.2, 12.6 Hz), and 5.51 (1H, dd, J = 12.6, 3.0 Hz) and 2.79 (1H, dd, J = 17.2, 3.0 Hz), 3.18 (1H, dd, J = 17.2, 12.6 Hz), and 5.53 (1H, dd, J = 12.6, 3.0 Hz) corresponding to protons at 2,3 and 2″,3″ positions of two flavanone moieties, indicating that compound **1** is a biflavanone derivative. The decoupled and DEPT ^{13}C NMR spectra displayed signals for all the 31 carbons, including two flavanone carbonyls and nine oxygen-bearing quaternary carbons, supporting the biflavanone structure of **1**. The ^{1}H NMR spectrum of **1** also displayed two downfield signals at δ 12.10 and 12.15, indicating the presence of two hydrogen-bonded hydroxyls at 5 and 5″ positions. A D_2O exchangeable signal at δ 9.10, integrating for two protons, indicated two more phenolic hydroxyls in **1**. A methoxyl singlet at δ 3.83 was placed at C-7 as it showed correlation with this carbon at 168.3 ppm in its HMBC spectrum and two strong NOE peaks with H-6 (δ 6.02) and H-8 (δ 6.04) in its NOESY spectrum (Figure 1). Four protons comprising two sets of *meta*-coupled doublets (J = 2.3 Hz) at δ 6.02 and 6.04 and at 5.94 and 5.96 correspond to 6,8 and 6″,8″ protons of rings A and D, respectively. The signals at δ 7.52 (d, J = 8.8 Hz) and 6.98 (d, J = 8.8 Hz), each integrating for two protons, showed the presence of a *para*-substituted aromatic ring in **1** and were assigned to 2‴,6‴ and 3‴,5‴ protons of ring E. A set of ABC-type aromatic proton signals at 7.10 (1H, d, J = 8.3 Hz), 7.26 (1H, d, J = 2.1 Hz), and 7.30 (1H, dd, J = 8.3, 2.1 Hz) were assigned to H-5′, H-2′, and H-6′ protons, respectively, of 3′,4′-disubstituted ring B. From ^{1}H−^{13}C long-range HMBC correlations (Figure 1), the two phenolic hydroxyls (δ 9.10) in **1** were located at C-4′ and C-7″ positions of ring B and ring D, respectively.

Apart from two carbonyl carbons, of the nine oxygen-bearing quaternary carbons in **1**, four carbons were attached to hydroxyl groups, two to pyranone oxygens, and one to a methoxyl group. This suggested that the remaining two oxygenated quaternary carbons should be involved in the interflavonoid ether linkage. Comparison of ^{13}C NMR spectral data of **1** with naringenin[13] (Table 1) showed that C-3′ of ring B should be involved in interflavonoid ether linkage[14] with C-4‴ of ring E, as the resonance of C-3′ was shifted downfield by 27.4 ppm from the corresponding carbon resonance of naringenin. The HMBC spectrum of **1** further confirmed the involvement of C-3′ and C-4‴ in interflavonoid ether linkage, as these carbons showed correlations with H-2′, H-5′, and OH-4′ and H-2‴, H-6‴, H-3‴, and H-5‴, respectively (Figure 1). The absolute configurations of C-2 and C-2″ were shown to be *S*,[15] as the CD spectrum of **1** exhibited positive and negative cotton effects at 329 and 292 nm, respectively. Thus from the foregoing spectral studies, compound **1** was characterized as 7-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone.

Experimental Section

General Experimental Procedures. Melting points were uncorrected. IR spectra were recorded in KBr disks on a Perkin-Elmer 283B spectrophotometer, and UV spectra with a Shimadzu UV-240 spectrophotometer. The CD spectrum was

* To whom correspondence should be addressed. Tel.: 91-8574-42471. Fax: 91-8574-27499/25211. E-mail: pcjobs@mail.com.
† Sri Venkateswara University.
‡ Dr. Reddy's Research Foundation.
§ Museum National d'Histoire Naturelle.

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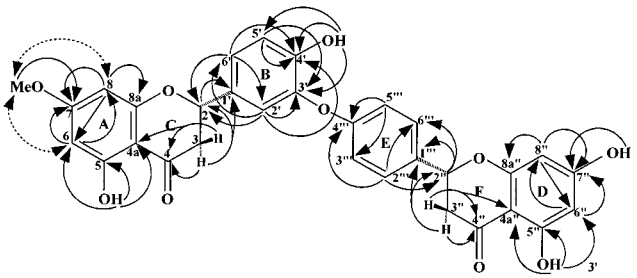

Figure 1. Selected HMBC (→) and NOESY (dashed ↔) correlations for **1**.

Table 1. ^{13}C NMR (75 MHz, Me_2CO-d_6, TMS) Data of **1** and Naringenin

carbon	**1**	naringenin	carbon	**1**
2	79.5	80.1	2″	79.4
3	43.5	43.7	3″	43.5
4	197.4	197.3	4″	196.9
4a	103.7	103.1	4a″	103.2
5	165.0	165.0	5″	165.3
6	95.6	97.0	6″	96.9
7	168.3	168.0	7″	167.4
8	94.6	96.1	8″	95.9
8a	164.0	164.5	8a″	164.2
1′	132.0	130.7	1‴	134.0
2′	121.0	128.8	2‴	129.0
3′	143.6	116.2	3‴	117.4
4′	150.5	158.5	4‴	159.2
5′	118.2	116.2	5‴	117.4
6′	124.9	128.8	6‴	129.0
7-OMe	56.2			

recorded in MeOH at 15 °C on JASCO J 715 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 MHz spectrometer in Me_2CO-d_6 and DMSO-d_6, with TMS as internal standard. EIMS were obtained on Nermag R 10−10 and Hewlett-Packard 5989X mass spectrometers at 70 eV by direct inlet probe. HRFABMS was obtained with a VGZAB-SEQ mass spectrometer using a thioglycerol matrix. HMBC and NOESY spectra were recorded using standard pulse sequences. Acme Si gel (finer than 200 mesh) was used for column chromatography.

Plant Material. The leaves of *O. beddomei* were collected in November 1997, in the Tirumala hills, South India. A voucher specimen (DG-973) was deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

Extraction and Isolation. Shade-dried and powdered leaves of *O. beddomei* (2.5 kg) were defatted with *n*-hexane and then extracted with Me_2CO, followed by MeOH. The Me_2CO extract was solvent fractionated with toluene and EtOAc. The EtOAc concentrate was column chromatographed over Si gel using C_6H_6, $CHCl_3$, EtOAc, and their mixtures as eluents. The $CHCl_3$ eluates, on further purification over a Si gel column using C_6H_6−$CHCl_3$ (2:8 and 1:9 gradients), yielded colorless needles (Me_2CO) of **1** (8 mg) and **2** (6 mg), respectively. The solid obtained from $CHCl_3$−EtOAc (9:1) eluates on crystallization from MeOH afforded pale yellow needles of **3** (100 mg). The $CHCl_3$−EtOAc (7:3 and 6:4) eluates on concentration followed by crystallization from MeOH gave yellow needles of **4** (20 mg) and **5** (50 mg), respectively. Purification of $CHCl_3$−EtOAc (2:8) eluates on a Si gel column employing hexane−EtOAc step gradient yielded greenish yellow needles (MeOH) of **6** (30 mg), colorless needles (Me_2CO) of **7** (20 mg), yellow crystals (MeOH) of **8** (40 mg), and colorless needles (Me_2CO) of **9** (20 mg). The EtOAc eluates on concentration gave a yellow solid that, on crystallization from MeOH, afforded yellow needles of **10** (30 mg).

7-*O*-Methyltetrahydroochnaflavone (1): colorless needles (Me_2CO), mp 170−172 °C; $[\alpha]^{25}_D$ −6.60° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 285 (4.30), 330 (3.45) nm; CD (MeOH) λ nm ($\Delta\epsilon$) 292 (−2.60), 329 (+0.66); IR (KBr) ν_{max} 3427, 1641, 1610, 1510, 1464, 1300, 1157 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 300 MHz) δ 12.15 (1H, s, OH-5″), 12.10 (1H, s, OH-5), 9.10 (2H, s, OH-4′ and OH-7″), 7.52 (2H, d, J = 8.8 Hz, H-2‴ and H-6‴), 7.30 (1H, dd, J = 8.3, 2.1 Hz, H-6′), 7.26 (1H, d, J = 2.1 Hz, H-2′), 7.10 (1H, d, J = 8.3 Hz, H-5′), 6.98 (2H, d, J = 8.8 Hz, H-3‴ and H-5‴), 6.04 (1H, d, J = 2.3 Hz, H-8), 6.02 (1H, d, J = 2.3 Hz, H-6), 5.96 (1H, d, J = 2.3 Hz, H-8″), 5.94 (1H, d, J = 2.3 Hz, H-6″), 5.53 (1H, dd, J = 12.6, 3.0 Hz, H-2″), 5.51 (1H, dd, J = 12.6, 3.0 Hz, H-2), 3.83 (3H, s, 7-OMe), 3.20 (1H, dd, J = 17.2, 12.6 Hz, H-3$_{ax}$), 3.18 (1H, dd, J = 17.2, 12.6 Hz, H-3″$_{ax}$), 2.81 (1H, dd, J = 17.2, 3.0 Hz, H-3$_{eq}$), 2.79 (1H, dd, J = 17.2, 3.0 Hz, H-3″$_{eq}$); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 556 [M]$^+$ (6) 539 (2), 446 (1), 404 (3), 390 (4), 389 (15), 364 (2), 301 (1), 285 (1), 271 (1), 255 (1), 238 (3), 225 (5), 193 (5), 167 (18), 153 (11), 140 (100); HRFABMS *m*/*z* 557.1453 [M + H]$^+$ (calcd for $C_{31}H_{25}O_{10}$, 557.1448).

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