Two New Biflavonoids from *Ochna obtusata*

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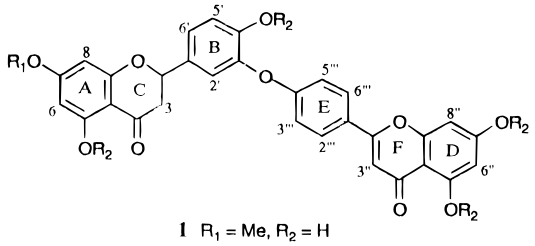
The leaves of *Ochna obtusata* (Ochnaceae) afforded two new biflavonoids together with the known compounds ochnaflavone, kaempferol 3-*O*-glucoside, and quercetin 3-*O*-glucoside. The new biflavonoids were characterized as 2,3-dihydroochnaflavone 7-*O*-methyl ether (**1**) and 2,3-dihydroochnaflavone (**2**) by spectral and chemical transformation studies.

Ochna obtusata DC (Ochnaceae) is a medium-sized tree found widely throughout the hilly tracts of South India.[1] The genus *Ochna* is rich in biflavonoids,[2−6] and some of the members of this genus are extensively used in traditional Indian medicine[1,7] in the treatment of epilepsy, mentrual complaints, lumbago, asthma, ulcers, and as an antidote to snake bites. As there is no record of any phytochemical work on *O. obtusata*, we have examined the leaves of this species and report the isolation and characterization of two new biflavonoids, **1** and **2**, besides three known compounds, ochnaflavone, kaempferol 3-*O*-glucoside, and quercetin 3-*O*-glucoside.

The acetone extract of *O. obtusata* was chromatographed over a Si gel column to yield two new biflavonoids, **1** and **2**. The color reactions and UV absorption maxima (292 and 322 nm) of **1** and **2** were very similar, which suggested that both compounds possessed an identical flavonoid system. The molecular ion of **1** at *m/z* 554 ($C_{31}H_{22}O_{10}$) showed it to be a methyl derivative of **2** (*m/z* 540, $C_{30}H_{20}O_{10}$).



1 R_1 = Me, R_2 = H
2 R_1 = R_2 = H
3 R_1 = Me, R_2 = Ac
4 R_1 = R_2 = Ac

The ^{13}C NMR spectrum of compound **1** showed signals for all 31 carbons of the molecule including three sp^3 carbons and 28 sp^2 carbons, which include signals for two carbonyl groups at 197.3 and 183.0 ppm. The IR spectrum exhibited a broad OH absorption band at 3168 and a chelated carbonyl band at 1648 cm^{-1}. The latter was resolved into two bands at 1683 and 1648 cm^{-1} in the IR spectrum of the tetraacetate of **1** (**3**) ([M]$^+$ 722). Such spectral changes on acetylation are reminiscent of the behavior of flavanone and flavone systems bearing hydroxyl groups at C-5.[8] These observations and selective molecular mass of 554 hinted that compound **1** could be a flavanone-flavone based biflavonoid.

The ^1H NMR spectra of **1** and its acetate (**3**) (Table 1) proved the presence of four hydroxyl groups in compound **1** of which two hydroxyl resonances appeared in the downfield region at δ 12.03 and 12.93, indicating the presence of chelated hydroxyls at the 5 and 5″ positions. The ^1H NMR spectrum of **1** showed a one-proton singlet at δ 6.69 and three double doublets at δ 5.54, 3.32, and 2.83, characteristic of a flavone and flavanone unit,[9] respectively. Four protons comprising two sets of *meta*-coupled doublets appearing at δ 6.05 and 6.02 (J = 2.4 Hz) and 6.25 and 6.53 (J = 2.1 Hz) were assigned to the 6, 8, 6″, and 8″ protons of ring A and ring D of the flavanone and flavone moieties, respectively. This implies that these carbons are not involved in interflavonoid linkage. The signals at δ 7.14 (d, 1H, J = 8.8 Hz), 7.34 (d, 1H, J = 2.2 Hz), and 7.36 (dd, 1H, J = 8.8, 2.2 Hz) correspond to 5′, 2′, and 6′ protons of ring B of the flavanone moiety. Further, the ^1H NMR spectrum also showed the presence of a set of A_2B_2-type doublets (J = 9.0 Hz) at δ 8.03 and 7.08 and were assigned, respectively, to the 2‴, 6‴ and 3‴, 5‴ protons of ring E of the flavone moiety. The remaining signal at δ 3.83 integrating for three protons indicated the presence of a methoxyl group. It showed ^3J coupling with C-7 at 168.8 ppm in its HMBC spectrum, which fixes its attachment to C-7 of ring A of the flavanone moiety. From $^1H-^{13}C$ long-range correlations, the two nonchelated hydroxyls at δ 8.76 and 9.62 are located at the C-4′ and C-7″ positions of ring B and ring D, respectively.

The above spectral studies suggested that compound **1** could be a biflavonoid with a C−O−C linkage since only nine out of 10 oxygen atoms in **1** have been accounted for by the presence of two chelated hydroxyls, two nonchelated hydroxyls, one methoxyl, two pyranone, and two pyrone oxygen atoms. Comparison of the ^{13}C NMR spectral data of **1** with naringenin and chrysin[10] (Table 1) showed that C-3′ of ring B and C-4‴ of ring E in **1** are involved in interflavonoid ether linkage[11] as the resonances of these carbons have shifted downfield by 27.5 and 31.5 ppm, respectively, from the corresponding carbon resonances of naringenin and chrysin. The HMBC spectrum of **1** further confirmed the in-

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Table 1. NMR Data (^{1}H-, 300.13 MHz, and ^{13}C-, 75.43 MHz, Me_2CO-d_6, TMS) for Compounds **1**, **2**, Naringenin, and Chrysin

	compd				
	1		**2**		naringenin
position	δ_C (ppm)	δ_H (ppm) m (J (Hz))	δ_C (ppm)	δ_H (ppm) m (J (Hz))	δ_C (ppm)
2	79.4	5.54 dd (12.7, 3.0)	79.3	5.52 dd (12.7, 3.0)	78.4
3	43.4	3.22 dd (17.1, 12.7)	43.5	3.19 dd (17.2, 12.7)	42.0
		2.83 dd (17.1, 3.0)		2.81 dd (17.2, 3.0)	
4	197.3		196.9		196.2
4a	103.7		103.2		101.8
5	165.1	12.03 s (OH)	166.3	12.13 s (OH)	163.6
6	95.5	6.05 d (2.4)	96.9	5.94 d (2.2)	95.9
7	168.8		167.4	9.68 s (OH)	166.7
7-OMe	56.0	3.83 s			
8	94.6	6.02 d (2.4)	95.9	5.96 d (2.2)	95.0
8a	163.9		164.1		162.9
1′	132.1		132.3		128.9
2′	121.5	7.34 d (2.2)	121.5	7.34 d (2.2)	128.2
3′	142.7		142.8		115.2
4′	150.6	8.76 s (OH)	150.5	8.81 s (OH)	157.8
5′	118.4	7.14 d (8.8)	118.4	7.13 d (8.6)	115.2
6′	125.5	7.36 dd (8.8, 2.2)	125.5	7.35 dd (8.6, 2.2)	128.2
					chrysin
2″	164.4		164.4		163.0
3″	105.0	6.69 s	105.1	6.68 s	105.0
4″	183.0		183.1		181.6
4a″	105.3		105.4		103.9
5″	163.3	12.93 s (OH)	163.4	12.92 s (OH)	161.5
6″	99.8	6.25 d (2.1)	99.9	6.26 d (2.4)	98.9
7″	164.9	9.62 s (OH)	165.0	9.73 s (OH)	164.3
8″	94.8	6.53 d (2.1)	94.8	6.53 d (2.4)	94.0
8a″	158.8		158.8		157.3
1‴	125.9		126.0		131.7
2‴	129.1	8.03 d (9.0)	129.1	8.03 d (8.8)	126.1
3‴	117.4	7.08 d (9.0)	117.5	7.10 d (8.8)	128.9
4‴	162.1		162.1		130.6
5‴	117.4	7.08 d (9.0)	171.5	7.10 d (8.8)	128.9
6‴	129.1	8.03 d (9.0)	129.1	8.03 d (8.8)	126.1

volvement 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. Thus, from the foregoing spectral studies, compound **1** was characterized as 2,3-dihydroochnaflavone 7-*O*-methyl ether (**1**).

The ^{1}H and ^{13}C NMR spectra of compound **2** closely resemble that of **1** (Table 1) except for the appearance of a hydroxyl group at C-7 instead of a methoxyl group, which clearly showed that compound **2** could be a demethyl derivative of **1**. The proposed structure of compound **2** as 2,3-dihydroochnaflavone was confirmed by dehydrogenation[12] of its pentaacetate (**4**) to ochnaflavone pentaacetate.[3]

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at room temperature in $CHCl_3$. IR spectra were recorded in KBr disks on a Perkin-Elmer 283 B spectrophotometer and UV spectra with a Shimadzu UV-240 spectrophotometer. Mass spectra were recorded on a Nermag Sidar V 3.1 in the EI mode at 70 eV. ^{1}H and ^{13}C NMR spectra were determined on Bruker AM-300.13 or Varian 200 spectrometers equipped with 5 mm ^{1}H and ^{13}C probes, operating at 300.13 and 75.43 MHz or 200 and 50.4 MHz, respectively. Samples were run in Me_2CO-d_6 or $CDCl_3$, and chemical shifts were referenced to internal TMS (0.00 ppm).

Plant Material. *O. obtusata* DC. (Ochnaceae) leaves were collected from Talakona, near Tirupati, Andhra Pradesh, India, in 1993. A voucher specimen, KMC-981, is on deposit in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

Extraction and Isolation. Shade-dried and pulverized leaves (5 kg) of *O. obtusata* were successively extracted with petroleum ether, C_6H_6, and Me_2CO. Concentration of the Me_2CO extract gave 200 g of a dark green syrupy mass. It was solvent fractionated with toluene, EtOAc, and EtCOMe. The latter two fractions were found to be similar on TLC and hence combined. The combined fraction on concentration under reduced pressure gave a dark green solid (70 g), which was subjected to column chromatography over Si gel (700 g) and gradient elution with C_6H_6, C_6H_6/EtOAc, EtOAc, and EtOAc/MeOH mixtures. A total of 142 fractions (100 mL each) were collected and combined on the basis of TLC patterns.

Fractions 13−24, eluted with C_6H_6−EtOAc (9:1), were evaporated, and the resulting pale yellow residue (150 mg) was crystallized from MeOH to give 2,3-dihydroochnaflavone 7-*O*-methyl ether (**1**) as pale yellow needles (140 mg): mp 253−254 °C; R_f 0.96 (C_6H_6−C_5H_5N−HCO_2H, 36:9:5). Fractions 49−60, eluted with C_6H_6−EtOAc (1:1), were evaporated, and the yellow residue (135 mg) obtained was crystallized from MeOH to give 2,3-dihydroochnaflavone (**2**) as yellow needles (120 mg): mp 257−258 °C; R_f 0.87 (C_6H_6−C_5H_5N−HCO_2H, 36:9:5). Fractions 83−94, eluted with EtOAc, were evaporated, and the yellow residue (80 mg) was

crystallized from MeOH to give ochnaflavone as pale yellow needles (75 mg): mp 234−235 °C (lit.[3] mp 233−235 °C). Fractions 95−118, eluted with EtOAc−MeOH (9:1 and 8:2), were separated by PTLC (SiO_2, *n*-BuOH−HOAc−H_2O, 8:1:1) to yield kaempferol 3-*O*-glucoside as yellow crystals (70 mg), mp 178 °C, and quercetin 3-*O*-glucoside as yellow crystals (40 mg), mp 218 °C.

2,3-Dihydroochnaflavone 7-*O*-methyl ether (1): pale yellow needles (MeOH); mp 253−254 °C; $[\alpha]^{28}_D$ −20° (*c* 0.21, $CHCl_3$); UV (MeOH) λ max (log ϵ) 292 (4.78), 322 (4.70) nm; IR (KBr) ν_{max} 3168, 1648, 1584, 1536, 1512, 1440, 1316, 1168, 1040, 840 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* $[M]^+$ 554.1209 (calcd 554.1206 for $C_{31}H_{22}O_{10}$: EIMS (70 eV) *m/z* $[M]^+$ 554 (22), 539 (10), 526 (3), 525 (10), 415 (2), 405 (3), 402 (2), 388 (8), 387 (7), 375 (13), 362 (32), 301 (2), 286 (47), 285 (19), 269 (4), 254 (7), 253 (7), 241 (5), 213 (5), 193 (18), 167 (42), 153 (55), 138 (19), 121 (22), 109 (8), 95 (46), 89 (11), 69 (90), 57 (85), 44 (100), 41 (47).

Tetraacetate of 1 (3). Acetylation of **1** (10 mg) with Ac_2O (1.5 mL) and pyridine (0.3 mL) at room temperature for 48 h resulted in a white amorphous solid that on crystallization from EtOAc−$CHCl_3$ (1:1) afforded colorless needles: mp 225−227 °C; IR (KBr) ν_{max} 2852, 1773, 1683, 1648 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.85 (d, 2H, *J* = 8.2 Hz, H-2‴, 6‴), 7.32 (d, 1H, *J* = 8.0 Hz, H-5′), 7.22 (m, 2H, H-2′,6′), 7.10 (d, 2H, *J* = 8.2 Hz, H-3‴,5‴), 6.80 (d, 1H, *J* = 2.1 Hz, H-8″), 6.58 (s, 1H, H-3″), 6.49 (d, 1H, *J* = 2.1 Hz, H-6″), 6.39 (d, 1H, *J* = 2.2 Hz, H-6), 6.25 (d, 1H, *J* = 2.2 Hz, H-8), 5.43 (dd, 1H, *J* = 12.7, 3.0 Hz, H-2), 3.84 (s, 3H, OMe-7), 2.95 (dd, 1H, *J* = 17.0, 12.7 Hz, H-3 *trans*), 2.73 (dd, 1H, *J* = 17.0, 3.0 Hz, H-3 *cis*), 2.43 (s, 3H, OAc-5″), 2.36 (s, 6H, OAc-5,7″), 2.18 (s, 3H, OAc-4′); HREIMS *m/z* $[M]^+$ 722.1541 (calcd 722.1535 for $C_{39}H_{30}O_{14}$).

2,3-Dihydroochnaflavone (2): pale yellow needles (MeOH); mp 257−258 °C; $[\alpha]^{28}_D$ −35.25° (*c* 0.30, $CHCl_3$); UV (MeOH) λ max (log ϵ) 292 (4.81), 322 (4.78) nm; IR (KBr) ν_{max} 3305, 1560, 1512, 1504, 1360, 1296, 1176, 1032, 994, 848, 1664, 1616 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m/z* $[M]^+$ 540 (33), 538 (13), 511 (10), 496 (2), 404 (5), 391 (5), 388 (18), 387 (12), 375 (14), 362 (13), 286 (11), 270 (5), 269 (5), 253 (7), 236 (8), 179 (10), 153 (19), 152 (18), 126 (55), 111 (19), 108 (12), 97 (25), 85 (30), 69 (100), 55 (58), 41 (28); HREIMS *m/z* $[M]^+$ 540.1054 (calcd 540.1050 for $C_{30}H_{20}O_{10}$).

Pentaacetate of 2 (4). The acetate of **2** (**4**), prepared by treating with Ac_2O and pyridine at room temperature for 48 h, was isolated as colorless needles from Me_2CO: mp 258−260 °C; IR (KBr) ν_{max} 1775, 1688, 1680, 1648 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.84 (d, 2H, *J* = 8.5 Hz, H-2‴, -6‴), 7.35 (d, 1H, *J* = 8.5 Hz, H-5′), 7.20 (m, 2H, H-2′, 6′), 7.10 (d, 2H, *J* = 8.5 Hz, H-3‴, -5‴), 6.86 (d, 1H, *J* = 2.5 Hz, H-8″), 6.83 (d, 1H, *J* = 2.5 Hz, H-6″), 6.70 (s, 1H, H-3″), 6.52 (d, 1H, *J* = 2.5 Hz, H-8), 6.49 (d, 1H, *J* = 2.5 Hz, H-6), 5.50 (dd, 1H, *J* = 12.5, 3.0 Hz, H-2), 3.00 (dd, 1H, *J* = 17.5, 12.5 Hz, H-3 *trans*), 2.82 (dd, 1H, *J* = 17.5, 3.0 Hz, H-3 *cis*) 2.46 (s, 3H, OAc-5″), 2.38 (s, 6H, OAc-5, 7″), 2.32 (s, 3H, OAc-7), 2.20 (s, 3H, OAc-4′); HREIMS *m/z* $[M]^+$ 750.1588 (calcd 750.1584 for $C_{40}H_{30}O_{15}$).

Dehydrogenation of **4** with KOAc and iodine in glacial AcOH yielded colorless shiny needles in $CHCl_3$: mp 239−240 °C. The physical characteristics and spectral data of the dehydrogenated product were identical in all respects with ochnaflavone pentaacetate.[3]

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