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HIGHLY OXYGENATED FLAVONOIDS FROM *MURRAYA PANICULATA*

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Key Word Index—*Murraya paniculata*; Rutaceae; leaves; flavonoids; polymethoxyflavonoid; chemotaxonomy.

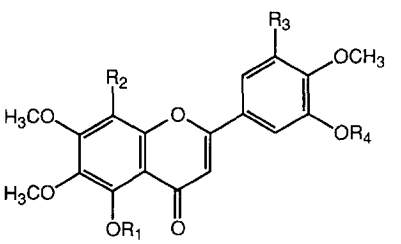
Abstract—Eight highly oxygenated flavones, including one new compound, were isolated from the leaves of an Indonesian medicinal plant, *Murraya paniculata*. Seven of them were identified as 5-hydroxy-6,7,8,3′,4′,5′-hexamethoxyflavone (gardenin A), 5,3′-dihydroxy-6,7,8,4′,5′-pentamethoxyflavone (gardenin C), 6,7,8,4′-tetramethoxy-5,3′,5′-trihydroxyflavone (gardenin E), 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone (5-*O*-desmethylnobiletin), 6,7,8,3′,4′,5′-hexamethoxyflavone, 5-hydroxy-6,7,3′,4′,5′-pentamethoxyflavone (umhengerin), 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavone either by direct comparison with authentic samples or by comparing their melting points and spectroscopic data with those reported in the literature. The new compound was elucidated as 5,3′,5′-trihydroxy-6,7,4′-trimethoxyflavone on the basis of chemical and spectroscopic studies.

INTRODUCTION

Murraya paniculata is a shrub distributed widely in tropical and subtropical Asia. Its leaves and roots have traditionally found wide medicinal uses in south east Asia and China. In Indonesia, the leaves of this species (local name: Daun kumning) are considered to be astringent and useful for the treatment of venereal disease. The first author has been engaged in chemical investigations on the leaves [1] and roots [2, 3] of *M. paniculata* collected in Taiwan and the root bark [4] of the same plant collected in Indonesia, and has reported the isolation of alkaloids [2, 3], polymethoxyflavones and prenylcoumarins [1, 4]. These chemical studies indicated that there is significant chemical difference between Formosan and Indonesian *M. paniculata*. Since the leaves of Indonesian *M. paniculata* have not been investigated chemically, we have undertaken a chemical study. In this report, we describe the isolation of flavones with 5,6,7,8-tetraoxygenated and 5,6,7-trioxygenated A-ring systems, and discuss their chemotaxonomic significance in the genus *Murraya*.

RESULTS AND DISCUSSION

The $CHCl_3$ extract of *M. paniculata* leaves collected in Indonesia was separated by a combination of silica gel, Sephadex LH-20 and reversed-phase silica gel column chromatography to afford eight compounds (**1**–**8**) in crystallized forms. Their spectroscopic data showed that they are highly oxygenated flavone derivatives. Compounds **1**–**4** had a fully oxygenated A-ring



1 R_1=R_3=H, R_2=OCH_3, R_4=CH_3
2 R_1=H, R_2=R_3=OCH_3, R_4=CH_3
3 R_1=H, R_2=OCH_3, R_3=OH, R_4=CH_3
4 R_1=R_4=H, R_2=OCH_3, R_3=OH

5 R_1=CH_3, R_2=H, R_3=OCH_3, R_4=CH_3
6 R_1=R_2=H, R_3=OCH_3, R_4=CH_3
7 R_1=R_2=H, R_3=OH, R_4=CH_3
8 R_1= R_2=R_4=H,, R_3=OH

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(5,6,7,8-tetraoxygenated) system, whereas compounds **5–8** have a 5,6,7-trioxygenated A-ring system. All compounds possessed a 3′,4′,5′-trioxygenated B-ring system, except for compound **1** where the B-ring was 3′,4′-dioxygenated.

Compound **1** was readily identified as 5-*O*-desmethylnobiletin, a flavone widely known from the genus *Citrus*, by comparison of its spectral values with those of the corresponding authentic compounds reported in the literature [5]. All the spectral data (UV, IR and ^{1}H NMR) and mps for compounds **2–4** corresponded to those of gardenins A [6], C [6] and E [6], respectively. These three compounds were isolated first from bud exudates (dikamali gum) of *Gardenia lucida* (Rubiaceae) [7] and were later found to occur in *Tamarix dioica* (Tamaricaceae) [6]. On methylation with diazomethane, compounds **6–8** afforded a hexamethoxyflavone, which was found to be identical to compound **5**. The spectral data of compound **5** were in good agreement with those of 5,6,7,3′,4′,5′-hexamethoxyflavone [8, 9], though the reported mps [8, 9] were much lower than that of **5**. Compound **6** was identified as 5-hydroxy-6,7,3′,4′,5′-pentamethoxyflavone (umhengerin), an antimicrobial flavonoid isolated from *Lantana trifolia*, by comparison of the spectral data with those reported in the literature [10]. The ^{1}H NMR spectrum of compound **7** showed that it contained an unsymmetrical 4′,5′-dimethoxy-3′-hydroxyphenyl system [δ 6.95 (*d*, $J = 2.1$ Hz, 2′-H) and 7.18 (*d*, $J = 2.1$ Hz, 6′-H)], as in gardenin C (**3**). This compound was finally identified as 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavone by direct comparison with a synthetic sample [11]. The isolation of a flavone with nominally the same structure was claimed from *Carphochaete bigelovii* (Asteraceae) [12]. However, the structure of this flavone should be re-examined on the following grounds. Proton signals at the 2′- and 6′-positions were observed as an equivalent singlet peak, which was inconsistent with the unsymmetrical substitution pattern of its B-ring. A large UV bathochromic shift (40 nm) by the addition of NaOMe is supportive of assigning its B-ring hydroxyl to the 4′-position rather than to the 3′-position. Moreover, this sample appeared to have been obtained in uncrystallized forms and, thus, its purity is questionable. Hence, this is to be the first authentic report of the isolation of 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavone from a natural source. Compound **8** was a new 5,6,7,3′,4′,5′-hexaoxygenated flavone possessing three methoxyls and three hydroxyls. The symmetrical B-ring system was indicated by the presence of a singlet peak with an integration of two protons at δ 7.11 assignable to the 2′- and 6′-positions. The presence of a chelated 5-OH was indicated by a sharp singlet at δ 12.87 in the ^{1}H NMR that disappeared on deuteration and also a large batochromic shift (29 nm) by the addition of $AlCl_3$ in the UV spectrum. The absence of significant bathochromic shifts by both NaOMe and NaOAc shift reagents in the UV spectrum. The absence of significant bathochromic shifts by both NaOMe and NaOAc shift reagents in the UV spectrum revealed the absence of free hydroxyls at the 4′- and 7-positions. From the above evidence the structure of compound **8** was elucidated as 5,3′,5′-trihydroxy-6,7,4′-trimethoxyflavone.

Although some polymethoxyflavonoids have been used as chemotaxonomic markers in some rutaceous genera, e.g. *Citrus* [13], they generally occur as minor constituents in the genus *Murraya*. As cited previously [1–4], *M. paniculata* has long been subjected to extensive chemical investigations and coumarin derivatives have been shown to be predominant secondary metabolites in this plant. However, as shown in this paper, Indonesian *M. paniculata* is distinctive due to the presence of characteristic flavones in high content. This feature was not found not only in *M. paniculata* at other localities but also in its taxonomic allies (T. Kinoshita, unpublished data). Thus, Indonesian *M. paniculata* might be regarded as a distinct chemical variety at the infraspecific level. It is also of interest to investigate whether infraspecific chemical variation is correlated with the geographical distribution of this species. As an additional remark, the voucher specimen of *M. paniculata* investigated chemically in this report is deposited in a complete form with flowers and leaves, but it is morphologically indistinguishable with those of the same plant species of other locality.

EXPERIMENTAL

General. Mps: uncorr. IR: KBr. UV: MeOH. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR: $CDCl_3$ or pyridine-d_5 with TMS as int. standard. Silica gel CC: Wakogel C-200, benzene–Me_2CO (B-A) or *n*-hexane–EtAcO. Sephadex LH-20 CC: Pharmacia, $CHCl_3$–MeOH (C–M). Reversed-phase CC: Merck RP-8, MeOH–H_2O (M–W). TLC silica gel ($60F_{254}$, Merck), spots were detected by inspection under UV light (254 and 365 nm) or by the colours developed after spraying with 10% H_2SO_4 followed by heating.

Plant material. Leaves of *M. paniculata* (L.) Jack were collected in 1984 through 1985 near Sukabumi, Java, Indonesia. A voucher specimen is deposited at the Herbarium of the University of Tokyo. Another sample was collected near Bandung, Java, Indonesia by the second author (K. F.). Since TLC analysis of both samples showed the same chemical spectrum, chemical work was undertaken with plant material collected in Sukabumi.

Extraction and isolation. Dried leaves (1 kg) of *M. paniculata* were extracted ×2 with $CHCl_3$ at room temp. and the combined extracts evapd to dryness under red. pres. to yield a greenish viscous syrup (98.9 g). The whole extract was dissolved in Me_2CO and adsorbed on silica gel (100 g). The adsorbed material was transferred to a silica gel column (650 g) packed in *n*-hexane. The column was eluted with the following solvent system: *n*-hexane (4.5 l), *n*-hexane–EtAcO 10:1 (3 l), 5:1 (2 l), 4:1 (2 l), 3:2 (3 l), 1:1 (3 l) and Me_2CO (3 l). Frs of 500 ml each were

collected and combined into 12 frs on the basis of their TLC composition: FR. I (frs 1–18; >10 g); FR. II (frs 19–20; 4.58 g); FR. III (frs 21–22; 1.36 g); FR IV (frs 23–24; 0.45 g), FR. V (frs 25–29; 1.72 g); FR. VI (frs 30–32; 0.83 g); FR. VII (frs 33–34; 0.65 g); FR. VIII (frs. 35–37; 3.71 g); FR. IX (frs 38–42; 9.01 g); FR. X (frs 43–45; 3.64 g); FR. XI (frs 46–49; 3.95 g); FR XII (frs 50–53; 20.69 g). Frs VII–IX were obtained as semicrystalline solids and recrystallized from MeOH to furnish gardenin A (**2**) (2.90 g) (mp 156–158°, lit. [6] 161–162°). The mother liquor of FR. IX was subjected to Sephadex LH-20 CC (C–M 1:2 as eluant) followed by repeated silica gel CC on elution with B–A to give gardenin C (**3**) (0.79 g) (mp 181–182°, lit. [6] 179–180°). FR. X was subjected successively to Sephadex LH-20 (C–M 1:2) and silica gel (B–A) CC to afford 5-*O*-desmethylnobiletin (**1**) (0.49 g) (mp 144°, lit. [5] 144–146°). The CC separations of FR. XI over Sephadex LH-20 (C–M) and silica gel (B–A) furnished 5-hydroxy-6,7,3′,4′,5′-pentamethoxyflavone (**6**) (0.22 g) (mp 202–203°, lit. [9] 193–194°), gardenin E (**4**) (0.34 g) (mp 233–235°, lit. [6] 234°) and 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavone (**7**) (0.08 g). FR. XII was chromatographed over silica gel and separated by elution with a gradient solvent system of B–A increasing the amount of Me_2CO stepwise (47:3–17:8). Frs eluted with B–A (21:4) were combined and further subjected successively to LH-20 (C–M) and reversed-phase silica gel CC (M–W, 4:1) to give 5,6,7,3′,4′,5′-hexamethoxyflavone (**5**) (0.27 g). Combined frs eluted with B–A (4:1) afforded precipitates, which were recrystallized to give 5,3′,5′-trihydroxy-6,7,4′-trimethoxyflavone (**8**) (0.14 g).

5,6,7,3′,4′,5′-*Hexamethoxyflavone* (**5**). Needles from MeOH, mp 150–152° (lit. [8] 114°; lit. [9] 116–117°). IR ν_{max}^{KBr} cm^{-1}: 2951, 2840, 1642, 1601, 1468, 1451, 1350, 1256, 1121. UV λ_{max}^{MeOH} nm (log ε): 232 sh (4.39), 266 (4.15), 316 (4.47). ^1H NMR (400 MHz, $CDCl_3$): δ 3.93 (6H, *s*, 2 × OMe), 3.96 (6H, *s*, 2 × OMe), 4.00 4.01 (3H each, *s*, OMe), 6.61 (1H, *s*, 3-H), 6.81 (1H, *s*, 8-H), 7.07 (2H, *s*, 2′- and 6′-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.3 (*q*), 56.4 (2 × C, *q*), 61.0 (*q*), 61.5 (*q*), 62.1 (*q*), 96.1 (*d*), 103.5 (2 × C, *d*), 108.2 (*d*), 112.9 (*s*), 126.8 (*s*), 140.4 (*s*), 141.0 (*s*), 152.6 (*s*), 153.5 (2 × C, *s*), 154.5 (*s*), 157.7 (*s*), 160.9 (*s*), 177.0 (*s*). EI-MS *m/z* (rel. int.): 402 ($[M]^+$, 30), 387 (100), 371 (9), 326 (7); HRMS: $[M]^+$ 402.1318 ($C_{21}H_{22}O_8$ requires 402.1315); *Analyt.* found: C, 62.79; H, 5.60 (calcd for $C_{21}H_{22}O_8$; C, 62.68; H, 5.51).

5,3′-*Dihydroxy*-6,7,4′,5′-*tetramethoxyflavone* (**7**). Pale yellow needles from Me_2CO, mp 219–221° (lit. [10] 216°). IR ν_{max}^{KBr} cm^{-1}: 3385, 2956, 2838, 1665, 1593, 1497, 1460, 1428, 1366, 1287, 1128, 1101. UV λ_{max}^{MeOH} nm (log ε): 233sh (4.30), 277 (4.26), 329 (4.39); $\lambda_{max}^{MeOH+MeONa}$ nm: 235sh, 264, 335; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 288, 358. ^1H NMR (400 MHz, $CDCl_3$): δ 3.93, 3.97, 3.98, 4.00, (3H each, *s*, OMe), 5.98 (1H, *br s*, 3′-OH, disappeared on deuteration), 6.55 (1H, *s*, 3-H), 6.59 (1H, *s*, 8-H), 6.95 (1H, *d*, J = 2.1 Hz, 2′-H), 7.18 (1H, *d*, J = 2.1 Hz, 6′-H), 12.68 (1H, *s*, 5-OH, disappeared on deuteration). ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.2 (*q*), 56.4 (*q*), 60.9 (*q*), 61.2 (*q*), 90.7 (*d*), 102.4 (*d*), 105.3 (*d*), 106.3 (*s*), 106.8 (*d*), 126.9 (*s*), 132.8 (*s*), 138.6 (*s*), 149.7 (*s*), 152.6 (*s*), 153.1 (*s*), 153.3 (*s*), 158.9 (*s*), 163.6 (*s*), 182.7 (*s*). EI-MS *m/z* (rel. int.): 374 ($[M]^+$, 100), 359 (79), 236 (32); HR-MS: $[M]^+$ 374.1002 ($C_{19}H_{18}O_8$ requires 374.1002); *Analyt.* found: C, 61.26; H, 4.80 (calcd for $C_{19}H_{18}O_8$; C, 60.96; H, 4.85).

5,3′,5′-*Trihydroxy*-6,7,4′-*trimethoxyflavone* (**8**). Pale yellow needles from MeOH, mp 291–293°. IR ν_{max}^{KBr} cm^{-1}: 3474, 2996, 2951, 2841, 1661, 1595, 1464, 1437, 1370, 1206, 1121. UV λ_{max}^{MeOH} nm (log ε): 276 (4.22), 328 (4.33); $\lambda_{max}^{MeOH+AcONa}$ nm: 277, 329; $\lambda_{max}^{MeOH+MeONa}$ nm: 241, 262, 338; $\lambda_{max}^{MeOH+AlCl3}$ nm: 287, 357. ^1H NMR (400 MHz, pyridine-d_5): δ 3.80, 3.90, 4.00 (3H each, *s*, OMe), 6.62 (1H, *s*, 3-H), 6.85 (1H, *s*, 8-H), 7.11 (2H, *s*, 2′- and 6′-H), 8.52 (2H, *br s*, 3′- and 5′-OH, disappeared on deuteration), 12.87 (1H, *br s*, 5-OH, disappeared on deuteration). ^{13}C NMR (100 MHz pyridine-d_5): δ 56.3 (*q*), 60.2 (*q*), 60.5 (*q*), 91.2 (*d*), 105.1 (*d*), 106.4 (*s*), 107.0 (2 × C, *d*), 127.3 (*s*), 133.0 (*s*), 140.5 (*s*), 152.8 (2 × C, *s*), 153.4 (*s*), 153.5 (*s*), 159.3 (*s*), 164.7 (*s*), 183.0 (*s*). EI-MS *m/z* (rel. int.): 360 ($[M]^+$, 100), 345 (79), 330 (20), 317 (13), 302 (12); HRMS: $[M]^+$ 360.0849 ($C_{18}H_{16}O_8$ requires 360.0845); *Analyt.* found: C, 60.48; H, 4.31 (calcd for $C_{18}H_{16}O_8$; C, 60.00; H, 4.48).

Methylation of compounds **6–8**. To a MeOH soln of each sample was added excess CH_2N_2–Et_2O and the mixt. kept at 0° for 24 hr. Excess CH_2N_2 was decomposed by adding HCO_2H and the solvent removed. The residue was recrystallized from MeOH. Each permethyl ether of compounds **6–8** was identical with **5** (mmp, IR and ^1H NMR).

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