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MYRICETIN 5,7,3′,4′,5′-PENTAMETHYL ETHER AND OTHER METHYLATED FLAVONOIDS FROM *MURRAYA PANICULATA*

TAKESHI KINOSHITA* and KURNIA FIRMAN†

Faculty of Pharmaceutical Sciences, Teikyo University, 1091-1 Suarashi, Sagamiko-machi, Tsukui-gun, Kanagawa 199-01, Japan; † Department of Pharmacy, Institut Teknologi Bandung, Jalan Ganeca 10, Bandung 40132, Indonesia

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Key Word Index—*Murraya paniculata*; Rutaceae; myricetin 5,7,3′,4′,5′-pentamethyl ether; flavonol; methoxyflavones; chemotaxonomy.

Abstract—Seven flavonoids, including one new flavonol, were isolated from the leaves of *Murraya paniculata* (Rutaceae). Four were identified as previously known constituents of *M. paniculata* or allied species, namely, 5,7,3′,4′,5′-pentamethoxyflavone, 5,7,8,3′,4′,5′-hexamethoxyflavone, 3,5,7,3′,4′,5′-hexamethoxyflavone and 3,5,7,8,3′,4′,5′-heptamethoxyflavone. The structure of the new flavonol was elucidated as 3-hydroxy-5,7,3′,4′,5′-pentamethoxyflavone on the basis of spectroscopic studies. Two other flavonoids, a methylated flavanone and its corresponding chalcone isomer were identified as 2-(*S*)-5,7,3′,4′,5′-pentamethoxyflavanone and 2′-hydroxy-3,4,5,4′,6′-pentamethoxychalcone, respectively.

INTRODUCTION

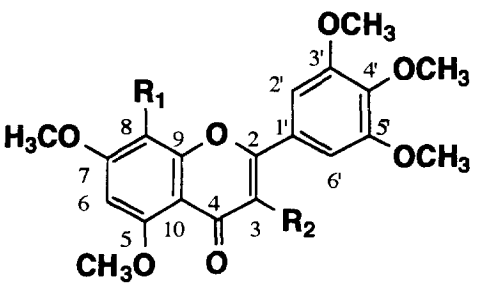
The dried leaves of *Murraya paniculata* (L.) Jack (Rutaceae) are frequently used in traditional Indonesian mixed herbal medicines (*jamu*). In a previous chemical investigation of the root of *M. paniculata* the first author reported an alkaloid, prenylated coumarins and polymethoxyflavones [1]. More recently, we reported the isolation of highly oxygenated flavonoids [2] and prenyl coumarins [3] from leaves of the same plant. Here, we describe the isolation and characterization of further minor components including a new flavonol.

RESULTS AND DISCUSSION

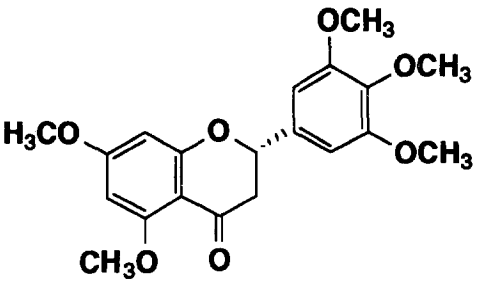
The chloroform extract of *M. paniculata* leaves was separated by a combination of silica gel and Sephadex LH-20 column chromatography to afford seven flavonoids. Two were readily identified by comparison with authentic markers of 5,7,3′,4′,5′-pentamethoxyflavone (**1**) and 5,7,8,3′,4′,5′-hexamethoxyflavone (**2**), which were previously obtained from the root of this plant [1]. Two other polymethoxyflavones were also isolated and characterized as 3,5,7,3′,4′,5′-hexamethoxyflavone (**3**) and 3,5,7,8,3′,4′,5′-heptamethoxyflavone (**4**). Compound **3**, a permethylated derivative of myricetin, has been reported from the leaves of *M. exotica* [4] and the flowers of *M. paniculata* [5], and compound **4** from the leaves of *M. paniculata* of Sri Lankan origin [6].

Compound **5** was obtained as pale yellow needles of mp 232–234 . Its molecular formula was determined to be $C_{20}H_{20}O_8$ based on the high resolution mass spectral analysis. Methylation of **5** with diazomethane afforded a hexamethoxyflavone identical to myricetin permethyl ether (**3**). The ^{1}H NMR spectrum of **3** exhibited the presence of five methoxyls, AB aromatic protons [δ 6.37 (*d*, J = 2.1 Hz) and 6.55 (*d*, J = 2.1 Hz)], one singlet at δ 7.49 with an integration of two protons, and a broad peak at δ 7.48 that disappeared on deuteration, but a diagnostic signal in a lower field (10–15 ppm) due to a chelated 5-hydroxyl group was not observed. The UV spectrum exhibited a large bathochromic shift (61 nm) on addition with $AlCl_3$. The above evidence assigned the structure of **5** as 3-hydroxy-5,7,3′,4′,5′-pentamethoxyflavone, and it structure was further substantiated by comparison of ^{13}C resonances of **5** with those of related compounds **1** and **3** as shown in Table 1. Minor constituents that are considered to be biogenetically related to these polymethoxyflavones were also obtained, and characterized as 2-(*S*)-5,7,3′,4′,5′-pentamethoxyflavanone (**6**) and 2′-hydroxy-3,4,5,4′,6′-pentamethoxychalcone (**7**). Compounds **6** and **7** have been reported previously in the Rutaceae, from *Neoraputia alba* [7] and *Mer-*

* Author to whom correspondence should be addressed.



1 R_1=R_2=H
2 R_1=OCH_3, R_2=H
3 R_1=H, R_2=OCH_3
4 R_1=R_2=OCH_3
5 R_1=H, R_2=OH



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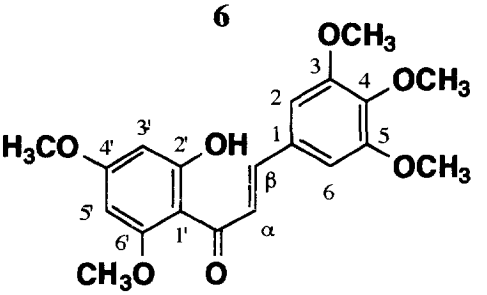

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rillia caloxylon [8], respectively. This is the second report of these constituents. The co-occurrence of **6** and **7** along with **1** and **3**, which have the same substitution pattern, is noteworthy in view of the biogenesis of polymethoxyflavonoids.

EXPERIMENTAL

General procedure. Mps: uncorr; IR: KBr; UV: MeOH; ^1H (400 MHz) and ^{13}C (100 MHz) NMR: $CDCl_3$ with TMS as int. standard; Si gel CC: Wakogel C-200. C_6H_6-Me_2CO (B-A); Sephadex LH-20 CC: Pharmacia, $CHCl_3$–MeOH; TLC: Si gel (60 GF_{254}, Merck), spots were detected by inspection under UV light (254 and 365 nm) or by the colours developed by spraying with 10% H_2SO_4 followed by heating.

Plant materials. The leaves of *Murraya paniculata* were collected in 1984–1985 near Sukabumi, Java, Indonesia. A voucher specimen is on deposit at the Herbarium of the University of Tokyo. A further 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 analysis was carried out with the plant material collected in Sukabumi.

Extraction and isolation. The $CHCl_3$ extract of *M. paniculata* leaves was separated into 12 frs (Fr. I–Fr. XII) by Si gel CC as previously described [2, 3]. Fr. XII (20.69 g) was chromatographed over Si gel (column size 6.5 × 18.5 cm) with C_6H_6–Me_2CO (B-A) as eluants increasing amounts of Me_2CO stepwise collecting 400 ml frs. Frs eluted with 6% B-A, on crystallization from MeOH, afforded 20 mg of 2′-hydroxy-3,4,5,4′,6′-pentamethoxychalcone (**7**), mp 186–187° (lit. [8] 176–178°). Frs eluted with 12% B-A were chromatographed over Sephadex LH-20 to yield 2-(*S*)-5,7,3′,4′,5′-pentamethoxyflavanone (**6**) (0.18 g), mp 174–175° (recryst. from MeOH), $[\alpha]_D$ −36.3° (MeOH; c 0.187). Frs eluted with 16% B-A were further separated by Sephadex LH-20 CC to afford 12 mg of 3-hydroxy-5,7,3′,4′,5′-pentamethoxyflavone (**5**) and 0.14 g of 5,7,8,3′,4′,5′-hexamethoxyflavone (**2**), mp 196–197° (lit. [1] 195–196°). Frs eluted with 20% B-A were separated by Sephadex LH-20 CC to yield 3,5,7,3′,4′,5′-hexamethoxyflavone (**3**) (0.96 g), mp 155–157° (lit [5] 148–150°). Frs eluted with 24% B-A were combined and recryst. from MeOH to afford 3,5,7,8,3′,4′,5′-heptamethoxyflavone (**4**) (0.36 g), mp 193–194° (lit. [6] 192–193°). Frs eluted with 32% B-A afforded ppts, which were recryst. from MeOH to give 5,7,3′,4′,5′-pentamethoxyflavone (**1**) (0.63 g), mp 195–196° (lit. [1] 197°).

3-Hydroxy-5,7,3′,4′,5′-pentamethoxyflavone (**5**). Yellow needles from Me_2CO, mp 232–234°; TLC: R_f on Si gel 60GF_{254} (Merck): 0.40 ($CHCl_3$-Me_2CO 4:1); 0.25 (benzene-EtOAc 4:1); spot colour (365 nm): dark yellow; $+NH_3$ (365 nm): pale yellow; UV λ_{max}^{MeOH} (log ε): 248 (4.05), 259sh (4.01), 305 (3.81), 355 (4.09); $\lambda_{max}^{MeOH+NaOAc}$: 248, 259sh, 305, 355; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$: 248, 259sh, 305, 355; $\lambda_{max}^{MeOH+MeONa}$: 259, 398; $\lambda_{max}^{MeOH+AlCl_3}$: 263sh, 272, 335, 416; $\lambda_{max}^{MeOH+AlCl_3+HCl}$: 263sh, 272, 335, 416 nm. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.93 (3H, *s*, 7-OMe), 3.94 (3H, *s*, 4′-OMe), 3.97 (6H, *s*, 3′- and 5′-OMe), 3.99 (3H, *s*, 5-OMe), 6.37 (1H, *d*, J = 2.1 Hz, 6-H), 6.55 (1H, *d*, J = 2.1 Hz, 8-H), 7.48 (1H, *br d*, 3-OH, disappeared on deuteration), 7.49 (2H, *s*, 2′- and 6′-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: see Table 1. EIMS *m/z* (rel. int., %): 388 (M^+, 100), 373 (37), 342 (27), 179 (10). HRMS: $[M]^+$ 388.1157 ($C_{20}H_{20}O_8$ requires 388.1159).

Table 1. ^{13}C NMR (100 MHz, δ ppm, TMS as internal standard) spectral data of flavonoids isolated from *M. paniculata* leaves

C*	1	3	5	6	C†	7
C-2	160.5	152.3	139.7	79.4	C-β	142.4
C-3	108.9	140.1	138.1	45.8	C-α	127.0
C-4	177.5	174.0	172.0	189.0	C = 0	192.4
C-5	161.0	161.1	160.7	162.3	C-6′	162.5
C-6	96.2	95.8	95.8	93.6	C-5′	91.4
C-7	164.1	164.0	164.6	166.0	C-4′	166.2
C-8	92.9	92.5	92.5	93.3	C-3′	93.9
C-9	159.9	158.8	158.9	164.9	C-2′	168.4
C-10	109.3	109.5	106.2	106.0	C-1′	106.4
C-1′	126.8	126.0	126.4	134.3	C-1	131.2
C-2′	103.5	105.9	105.1	103.3	C-2	105.7
C-3′	153.6	153.1	153.3	153.5	C-3	153.5
C-4′	140.9	141.6	141.7	138.3	C-4	140.2
C-5′	153.6	153.1	153.3	153.5	C-5	153.5
C-6′	103.5	105.9	105.1	103.3	C-6	105.7
3-OMe	--	60.1	--	--		
5-OMe	56.4	56.4	56.4	56.2	6′-OMe	55.8
7-OMe	55.8	55.8	55.9	55.6	4′-OMe	55.6
3′-OMe	56.4	56.4	56.4	56.2	3-OMe	56.2
4′-OMe	61.0	61.0	61.0	60.8	4-OMe	61.0
5′-OMe	56.4	56.4	56.4	56.2	5-OMe	56.2

Assignments were aided by ^{13}C–^{1}H COSY and long range ^{13}C–^{1}H COSY spectra.
* Numbering based on the flavone, flavonol or flavanone skeleton.
† Numbering based on the chalcone skeleton.

Methylation of **5**. To a MeOH soln of **5** (3 mg) 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 HCOOH, and then the solvent removed. The residue was recryst. from MeOH to give a methyl ether (2.5 mg) which was identical to myricetin permethyl ether (**3**) in mmp, IR and ^{1}H NMR.

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