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FLAVONOIDS FROM THE FRUITS OF *MURRAYA PANICULATA**

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Key Word Index—*Murraya paniculata*; Rutaceae; methoxyflavones; methoxyflavonols; biochemical systematics.

Abstract—In an investigation of the peel and pulp of the fresh ripe fruits of *Murraya paniculata* nine flavonoids: 5,7,3′,4′,5′-pentamethoxyflavanonol, 5,6,7,3′,4′,5′-hexamethoxyflavone, 3,5,6,7,3′,4′,5′-heptamethoxyflavone, 5,7,8,3′,4′,5′-hexamethoxyflavone, 3,5,7,8,3′,4′,5′-heptamethoxyflavone, 3,5,7,8,3′,4′-hexamethoxyflavone, 5-hydroxy-3,7,8,3′,4′-pentamethoxyflavone, 5-hydroxy-3,7,8,3′,4′,5′-hexamethoxyflavone and 8-hydroxy-3,5,7,3′,4′,5′-hexamethoxyflavone, were identified. The latter two compounds appear to be novel.

INTRODUCTION

Murraya paniculata (Linn.) Jack, which is native to Southeast Asia, was introduced to Brazil and has flourished in São Paulo state. In Brazil it is used as an ornamental plant, but this plant is used for its medicinal properties in tropical and subtropical Asia [1]. A search for previous phytochemical studies of *M. paniculata* revealed that only the coumarin, coumurrayin, has been reported from the ripe fruits of this species [2]. Working separately on peel and pulp of the fresh ripe fruits, we have isolated only polymethoxylated flavones, flavonols and flavanonols.

RESULTS AND DISCUSSION

The dichloromethane extract of the peel of *M. paniculata* after successive chromatographic separations afforded six flavonoids: 5,7,3′,4′,5′-pentamethoxyflavanonol [3, 4], 5,6,7,3′,4′,5′-hexamethoxyflavone (**1**) [5], 3,5,7,8,3′,4′,5′-heptamethoxyflavone (**2**) [6], 5,7,8,3′,4′,5′-hexamethoxyflavone (**3**) [7], 5-hydroxy-3,7,8,3′,4′,5′-hexamethoxyflavone (**4**) and 8-hydroxy-3,5,7,3′,4′,5′-hexamethoxyflavone (**5**). Compounds **4** and **5** appear to be novel.

The ^{13}C NMR data for **2** and **3** do not appear to have been reported in the literature. Thus, the ^{13}C chemical shifts of **2** and **3** have been listed in Table 1 using **1** and 5,7,8,2′,3′,4′,5′-heptamethoxyflavone (**6**) [3] as models. The carbon signals of **1**, isolated from *Neoraputia magnifica* (Rutaceae), were assigned by use of HMQC and HMBC experiments [5].

Compound **4** was obtained as a yellow powder. The presence of a flavonoid nucleus was suggested by its UV and IR spectra. In the ^1H NMR spectrum (Table 2) of **4**, three aromatic protons appeared at δ 6.43 (1H, *s*) and δ 7.52 (2H, *s*, H-2′ and H-6′), one hydroxyl group at δ 12.38 (1H, *s*, OH-5) and six methoxy groups at δ 3.95 (6H, *s*), 3.94 (6H, *s*), 3.92 (3H, *s*), and 3.90 (3H, *s*). The assignment of one singlet to H-2′ and H-6′ suggested that three methoxyls were attached to the B-ring. Furthermore, the singlet at δ 6.43 clearly indicated the A-ring to be 5,6,7- or 5,7,8-trisubstituted. A comparison of ^{13}C chemical shifts of 5-hydroxy-7,8- and 5-hydroxy-6,7-dimethoxyflavone and 5-hydroxy-3,7,8- and 5-hydroxy-3,6,7-trimethoxyflavonol indicated that the C-6 methine (*ca* δ 96) resonates at a lower field than the C-8 methine (*ca* δ 91) [3]. Thus, the correct A-ring structure was decided on the basis of the ^{13}C NMR spectrum (Table 1), which showed a signal at δ 95.5 for C-6, so placing the methoxyl substituents at C-7 and C-8. Moreover, the ^{13}C NMR spectrum showed signals for three methoxyl groups at δ 61.5, 61.0 and 60.3, indicating a 3-methoxyflavonol derivative and confirming the oxygenation patterns in the A and B-rings. This was corroborated by the fragments at *m/z* 207 $[C_{12}H_{14}O_4-Me]^+$ and 181 $[C_9H_8O_5-Me]^+$, associated with the retro-Diels–Alder cleavage of the C-ring followed by the loss of a methyl group, respectively. From these data **4** was characterized as 5-hydroxy-3,7,8,3′,4′,5′-hexamethoxyflavone. The structural assignment was also supported by comparison of the ^{13}C NMR spectrum with those of 4′,5-dihydroxy-3,3′,7,8-tetramethoxyflavone (ternatin, **7**) and 4′,5-dihydroxy-

* Based in part on the Ph.D. thesis presented by R.J.F. to the Universidade Federal de São Carlos.

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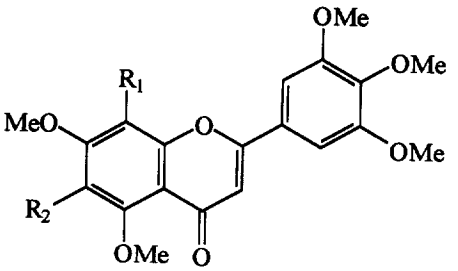
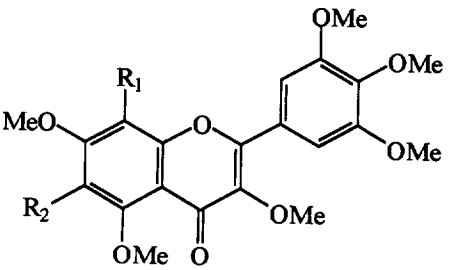


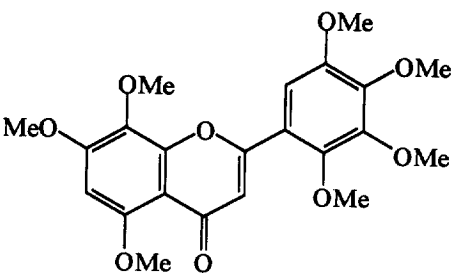
1: $R_1 = H$, $R_2 = OMe$

3: $R_1 = OMe$, $R_2 = H$



10: $R_1 = H$, $R_2 = OMe$

2: $R_1 = OMe$, $R_2 = H$



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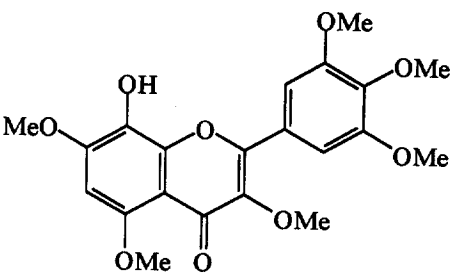

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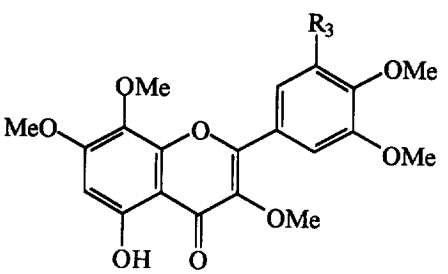
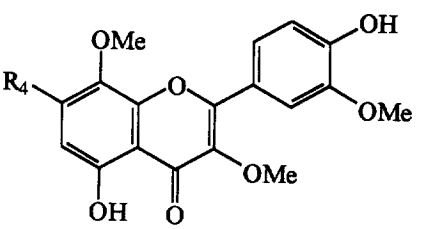


4: $R_3 = OMe$

9: $R_3 = H$



7: $R_4 = OMe$

8: $R_4 = OPre$

3,3′,8-trimethoxy-7-(3-methylbut-2-enyloxy)-flavone (**8**) [8]. Complete and unambiguous ^{13}C NMR assignments for **8** were made using the HMBC technique [8].

Compound **5** exhibited similar NMR spectra to **4** (Table 1 and 2) except for the absence of the signal for one chelated hydroxyl. The mass spectrum indicated a molecular formula $C_{21}H_{22}O_9$ ($[M]^+ = 418$), which strongly suggested an isomer of **4**. The ^{13}C NMR spectrum (Table 1) showed signals for two methoxyl groups at δ 61.3 and 60.1, which were assigned to the 3- and 4′- positions. This implies that the methoxyls in the A-ring were located at C-5 and C-7 (δ 56.4 and 56.1) and determined the position of the hydroxyl group at C-8. The 5,7,8- and 5,6,7-trimethoxyflavones can be differentiated by the ^{13}C NMR chemical shifts of the unsubstituted methine which absorbs at δ 92.6 (C-6, for example **6**) in the case of the former [3] and at δ 96.3 (C-8, for example **1**) in the case of the latter. Thus, the ^{13}C signal at δ 91.6 for C-6 supported the proposed structure of **5** as 8-hydroxy-3,5,7,3′,4′,5′-hexamethoxyflavone.

A hexane-soluble fraction of the hydromethanolic extract of pulp after successive chromatographic separations afforded three flavonols: **4**, **9** and **10**. Compound **4** could not be separated from a small amount of **9**. The ^1H NMR in addition to signals described above for **4**, revealed the presence of three protons in the B-ring, giving rise to an ABC coupling system (δ 7.02, *d*, $J = 8.8$ Hz, H-5′; δ 7.81, *d*, $J = 2.0$ Hz, H-2′, δ 7.86, *dd*, $J = 8.8$ and 2.0 Hz, H-6′). This was supported by the mass spectrum which showed fragments at *m/z* 177 $[C_{11}H_{12}O_3 - Me]^+$ and 181 $[C_9H_8O_5 - Me]^+$ due to the retro-Diels–Alder cleavage

Table 1. ^{13}C NMR chemical shift for compounds **2–5** and **10** and selected carbons in the model compounds **1** and **6–8**

C	1	2	3	6	4	7	8	5	10
2	161.0	156.1	160.2	158.0	155.1	156.9	156.8	156.1	157.7
3	108.3	139.6	107.8	112.3	138.8	139.2	139.2	137.7	140.1
4	177.2	174.0	177.8	177.7	179.0	179.8	179.8	172.3	173.7
5	154.5	151.6	151.9	151.8	158.5	158.3	158.2	150.8	153.5
6	140.4	91.9	92.3	92.6	95.5	96.6	97.7	91.6	140.2
7	157.8	156.2	156.6	156.3	157.4	159.4	158.8	156.8	157.8
8	96.3	130.0	130.5	130.5	128.8	129.8	130.1	130.3	96.0
9	152.6	150.5	156.3	156.1	147.1	149.2	149.4	141.6	152.4
10	112.9	108.8	108.7	106.0	105.0	105.2	106.2	105.9	113.1
1′	126.9	125.9	126.6	119.7	125.6	122.4	122.5	126.5	125.1
2′	103.4	105.2	103.1	147.2	105.9	112.9	112.9	104.6	105.8
3′	153.6	152.8	153.4	145.4	153.1	149.1	149.0	153.2	153.1
4′	140.9	141.0	140.7	149.2	140.1	152.1	152.1	139.3	141.1
5′	153.6	152.8	153.4	147.4	153.1	117.3	117.3	153.2	153.1
6′	103.4	105.2	103.1	106.0	105.9	123.9	123.9	104.6	105.8
OMe	56.4	55.8	55.9	56.0	56.2	56.3	56.1	56.1	56.4
OMe	56.4	55.8	56.1	56.1	56.2	56.9	60.5	56.1	56.4
OMe	56.4	56.1	56.2	56.4	56.4	60.4	61.9	56.1	56.4
OMe	61.1	56.2	56.5	61.0	60.3	61.9		56.4	60.1
OMe	61.6	59.7	61.0	61.1	61.0			61.0	61.0
OMe	62.2	62.0	61.4	61.2	61.5			61.3	61.6
OMe		62.4							62.3

Table 2. ^{1}H NMR chemical shift for compounds **4**, **5** and **9**

H	4	5	9
6	6.43 *s*	6.42 *s*	6.43 *s*
2′	7.52 *s*	7.61 *s*	7.81 *d* (2.0)
5′			7.02 *d* (8.8)
6′	7.52 *s*	7.61 *s*	7.86 *dd* (8.8, 2.0)
OH	12.38 *s*		12.47 *s*
OMe	3.90 *s*	3.92 *s*	3.89 *s*
OMe	3.92 *s*	3.93 *s*	3.91 *s*
OMe	3.94 *s*	3.95 *s*	3.93 *s*
OMe	3.94 *s*	3.95 *s*	3.96 *s*
OMe	3.95 *s*	3.95 *s*	3.98 *s*
OMe	3.95 *s*	4.02 *s*	

of the C-ring followed by loss of a methyl group, respectively. These data were consistent with the structure of 5-hydroxy-3,7,8,3′,4′-pentamethoxyflavone for **9**, which has been found previously in *Citrus reticulata* (tangerine peel) [9].

Compound **10** gave spectral data in agreement with those published for 3,5,6,7,3′,4′,5′-heptamethoxyflavonol [10]. However, its ^{13}C NMR data do not appear to have been reported previously in the literature. Thus, they have been listed in Table 1 using **1**, **2** and **3** as models.

A dichloromethane-soluble fraction of the methanolic pulp extract gave four flavonoids: **3**, **5**, 5,7,3′,4′,5′-pentamethoxyflavanonol [3, 4] and 3,5,7,8,3′,4′-hexamethoxyflavone [3, 11].

Other organs of *M. paniculata* have long been the subject of extensive chemical investigations, which have proved that it is a rich source of coumarins, flavones and flavonols as are other plants of the Aurantioideae [12]. The leaves have been reported to contain **2** [13], **3** [1, 13] and **10** [10], while **3** has also been found in the roots [7]. Flavonol **1** has been isolated from the leaves of *M. exotica* [6], which has been considered to be a synonym of *M. paniculata* [14]. By contrast, the flowers appear to be poor in flavonoids. However, the only flavonol, 3,5,7,3′,4′,5′-hexamethoxyflavone, reported from the flowers shows an oxidation level comparable to those of the leaves.

Flavonol **9**, 3,5,7,8,3′,4′-hexamethoxyflavone and 5,7,3′,4′,5′-pentamethoxyflavanonol are reported for the first time from the *Murraya*, while the latter does not appear to have been recorded previously from the Rutaceae.

EXPERIMENTAL

General. NMR: Bruker ARX 400, with TMS as int. standard; GC-MS: low resolution on a HP-2576 instrument; R-HPLC (recycling HPLC): model Shimadzu LC-6AD; the column used was a Shim-pack Prep-Sil (H), 250 × 20 mm, 5 μm particle size, 100 Å pore diameter; eluent: hexane–CH_2Cl_2–MeOH (5:15:0.3); flow rate: 3.0 ml min^{-1}; detection (Shimadzu SPD-6AV): UV λ 254 nm.

Plant material. Murraya paniculata was collected in São Carlos, SP, Brazil and vouchers are deposited at the Herbarium of Instituto de Biociências, USP, São Paulo.

Isolation of compounds. The seeds, peel and pulp of the fresh ripe fruits were sepd by a perforated plate sieve (0.5 mm; tyler 32). The seeds and peel were dried, powdered and successively extracted with hexane, CH_2Cl_2 and MeOH. The pulp was stirred with MeOH at room temp. The extract was decanted, and the residual pulp similarly extracted a second time. The combined extracts were evapd under vacuum and the residue partitioned into hexane-, CH_2Cl_2-, MeOH- and *n*-BuOH-soluble frs.

The concd CH_2Cl_2 extract of peel was submitted to vacuum chromatography over silica gel using hexane–Me_2CO (9:1), yielding 48 frs. Fr 23 was chromatographed on silica gel using hexane–CH_2Cl_2–MeOH (10:10:1) as eluents affording further frs. Fr 23-4 purified $\times 2$ by prep. TLC (silica gel; hexane–CH_2Cl_2–MeOH, 10:10:1) to yield a mixt. of **1** and 5,7,3′,4′,5′-pentamethoxyflavanonol (40 mg), which was sepd by R-HPLC (detection UV λ 254 nm) to give **1** (1st peak, 18 mg) and 5,7,3′,4′,5′-pentamethoxyflavanonol (2nd peak, 16 mg), after recycling $\times 3$/ Fr 33 was purified by prep. TLC as above to afford **2** (15 mg), **3** (13 mg) and **4** (15 mg). Fr 50 was purified as above affording **5** (20 mg).

The concd hexane-soluble pulp fr. was repeatedly purified by prep. TLC (silica gel; hexane–Me_2CO, 3:1) to yield **10** (20 mg) and a mixt. of **4** and **9** (15 mg).

The concd CH_2Cl_2-soluble pulp. fr. was subjected to CC over silica gel. Elution with a hexane–Me_2CO gradient afforded **5** (23 mg), 5,7,3′,4′,5′-pentamethoxyflavonol (20 mg), **3** (22 mg) and a mixt. of **2** and 3,5,7,8,3′,4′-hexamethoxyflavonol (38 mg) which was purified by RP-HPLC as above.

5-*Hydroxy*-3,7,8,3′,4′,5′-*hexamethoxyflavone* (**4**). Yellow plates; UV $\lambda_{max}^{CHCl_3}$ nm: 276, 309; IR ν_{max} (KBr) cm^{-1}: 3400, 1630. ^1H NMR (400 MHz, $CDCl_3$): Table 2; ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. MS *m/z* (rel. int.): 403 $[M-Me]^+$ (100), 207 (22), 181 (4).

8-*Hydroxy*-3,5,7,3′,4′,5′-*hexamethoxyflavone* (**5**). Amorphous solid; UV $\lambda_{max}^{CHCl_3}$ nm: 260, 358; IR ν_{max} (KBr) cm^{-1}: 3450, 1670. ^1H NMR (400 MHz, $CDCl_3$): Table 2; ^{13}C NMR (100 MHz, $CDCl_3$): Table 1. MS *m/z* (rel. int.): 418 $[M]^+$ (100), 403 $[M-Me]^+$ (43), 207 (62), 196 (6), 181 (25).

5-*Hydroxy*-3,7,8,3′,4′-*pentamethoxyflavone* (**9**). Yellow powder (trace of **9** with **4**). ^1H NMR (400 MHz, $CDCl_3$): Table 2. GC-MS of **9**: (GC: HP-1 column 50 m × 0.25 mm programmed from 100–200° at 8.0° min^{-1}) R_t 32.930 min. MS *m/z* (rel. int.): 388 $[M]^+$ (45), 373 $[M-Me]^+$ (100), 181 (6) 177 (7).

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