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A PYRANO CHALCONE AND A FLAVANONE FROM *NEORAPUTIA MAGNIFICA**

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Key Word Index—*Neoraputia magnifica*; Rutaceae; flavonoids; biochemical systematics.

Abstract—The hexane extract from the stems of *N. magnifica* var. *magnifica*, afforded two new flavonoids: 2′-hydroxy-3,4,4′,5-tetramethoxy-5′,6′-(2″,2″-dimethylpyran)chalcone and 5,6,7,3′,4′,5′-hexamethoxyflavanone which were identified on the basis of spectroscopic methods. The known flavonoids 2′-hydroxy-3,4,4′,5,6′-pentamethoxychalcone, 5,7,3′,4′,5′-pentamethoxyflavanone and 5,6,7,3′,4′,5′-hexamethoxyflavone, the sterols sitosterol and stigmasterol were also identified. These results do not seem to support the affiliation of *Neoraputia* to the tribe Cusparieae.

INTRODUCTION

Neoraputia magnifica (Engler) Emmerich is a tree found only in northwestern Brazil [1]. It was originally described by Engler as a species of *Raputia* Aublet. Emmerich later proposed the new genus *Neoraputia* Emmerich to accommodate six species, four of them form *Raputia* and two new ones. These two genera are assigned to the tribe Cusparieae [1]. No previous phytochemical work has been reported on *Raputia*. Recently, we have described the isolation and identification of eight polymethoxylated flavones and one flavanone from *N. alba* [2, 3]. Little is known about the chemistry of the *Neoraputia*, but, to date, it differs from other taxa of the tribe Cusparieae, notably by the absence of anthranilate alkaloids, coumarins and limonoids [4]. The extensive O-methylation of highly oxygenated flavones is a feature that is largely confined to the Citroideae [5]. Thus, our taxonomic interest in the Rutaceae stimulated an investigation of another plant of the *Neoraputia* genus, e.g. *N. magnifica* var. *magnifica* (Engler) Emmerich.

RESULTS AND DISCUSSION

The hexane extract from the stems of *N. magnifica* var. *magnifica*, afforded two new flavonoids **1** and **3**, besides three known flavonoids 2′-hydroxy-3,4,4′,5,6′-pentamethoxychalcone (**2**) [6], 5,7,3′,4′,5′-pentamethoxyflavanone (**4**) [2] and 5,6,7,3′,4′,5′-hexamethoxyflavone (**5**) [7], and a mixture of sitosterol and stigmasterol.

Compound **1** was isolated in very small amounts (0.7 mg) as a yellow powder. The UV absorption maximum at 286 and 352 nm suggested the presence of a chalcone [6]. The IR bands at 1620 and 3348 cm^{-1} were attributed to chelated carbonyl and phenolic hydroxyl group, respectively. Two doublets at δ 8.05 and 7.72 (J = 16.0 Hz) in the ^1H NMR spectrum (Table 1) revealed the AB system of a chalcone and a singlet at δ 14.24 corresponded to one chelated hydroxyl, thus attached to C-2′. The ^1H NMR spectrum also showed features of a chromene ring (δ 6.60 and 5.47 vinylic protons, and a singlet at δ 1.50, 6H, assigned to a pair of magnetically equivalent methyl group), and signals for four methoxyls. The assignment of one singlet to H-2 and H-6 (δ 6.86, 2H) suggested that three methoxyls are attached to B-ring. From the biosynthetic point of view the remaining methoxyl should be attached to C-4′ or C-6′ of the A-ring. Furthermore, a singlet at δ 6.07 (1H) clearly indicated the A-ring to be 2′,4′5′6′- or 2′,3′,4′,6′-tetrasubstituted. From the HMBC experiments (Table 2) the observed correlations between the hydroxyl proton at δ 14.24 and the ^{13}C signals at δ 105.9 (J^3), 167.9 (J^2) and 92.5 (J^3) led to their assignment as C-1′, C-2′ and C-3′, respectively, and thus indicating that the unsubstituted carbon must be vicinal to C-2′. The ^{13}C NMR spectrum (Table 3) showed a signal for only one methoxyl group attached to *ortho*-disubstituted carbon (δ 63.1) which was assigned to 4-OMe. This

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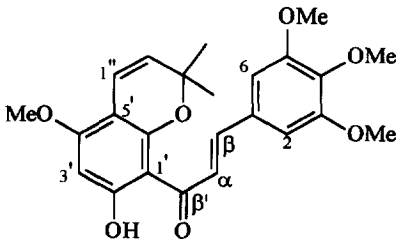

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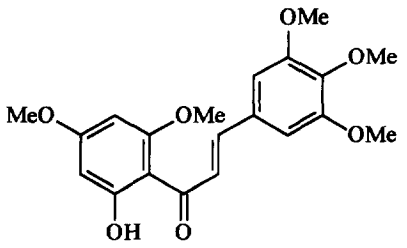

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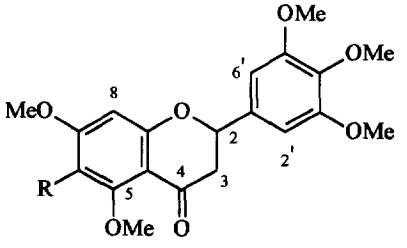
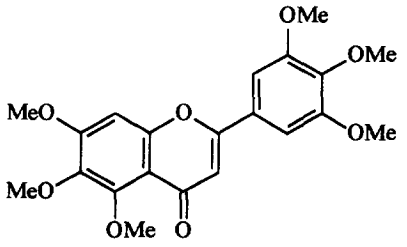


3: R = OMe

4: R = H



5

Table 1. ^{1}H NMR chemical shifts for compounds **1**–**5**

H	1	2	H	3	4	5
3′	6.07 *s*	6.12† *d* (2.4)	6		6.11 *d* (2.4)	
5′		5.97† *d* (2.4)	8	6.38 *s*	6.18 *d* (2.4)	6.81 *s*
2	6.86 *s*	6.84 *s*	2′	6.68 *s*	6.68 *s*	7.08 *s*
6	6.86 *s*	6.84 *s*	6′	6.68 *s*	6.68 *s*	7.08 *s*
β	7.72 *d* (16)	7.71 *d* (16)	2	5.34 *dd** (13.2; 2.8)	5.34 *dd** (13.2; 2.8)	
α	8.05 *d* (16)	7.80 *d* (16)	3	2.76 *dd** (16.4; 2.8)	2.76 *dd** (16.4; 2.8)	6.62 *s*
1″/2″	6.60 *d*/5.47 *d* (12)			3.03 *dd** (16.4; 13.2)	3.03 *dd** (16.4; 13.2)	
4″/5″	1.50 *s*					
OMe	3.87 *s*	3.84 *s*	OMe	3.88 *s*	3.84 *s*	3.93 *s*
OMe	3.90 *s*	3.90 *s*	OMe	3.90 *s*	3.84 *s*	3.93 *s*
OMe	3.90 *s*	3.91 *s*	OMe	3.90 *s*	3.84 *s*	3.96 *s*
OMe	3.91 *s*	3.92 *s*	OMe	3.90 *s*	3.86 *s*	3.96 *s*
OMe		3.92 *s*	OMe	3.91 *s*	3.87 *s*	4.00 *s*
OH	14.24 *s*		OMe	3.95 *s*		4.04 *s*

* The spectrum run in C_6D_6 was better resolved (**3**—H-2 δ 4.92, H_2-3 δ 2.76, 2.61; **4**—H-2 δ 4.97, H_2-3 δ 2.85, 2.66).

† Data obtained in this study suggest that these resonances were previously incorrectly assigned.

Table 2. HMBC for compounds **1**, **2**, **4** and **5**

	1		**2**		**4**		**5**
H/C		H/C		H/C		H/C	
2/	3; β; 4; 6	2/	3; β; 4; 6	2/	2′/6′	3/	2; 10
6/	5; β; 4; 2	6/	5; β; 4; 2	3 (2.76)/	4	8/	6; 7; 10
3′/	1′; 2′; 4′; 5′	3′/	1′; 2′; 4′; 5′	3 (3.03)/	2; 4	2′/	2; 4′; 6′
α (8.05)/	1; β′	α (7.80)/	1; β; β′	6/	7; 8; 10	6′/	2; 2′; 4′
β (7.72)/	2/6; β′	β (7.71)/	2/6; α; β′	8/	6; 7		
OH/	1′; 2′; 3′	OH/	1′; 2′; 3′	2′/	1′; 6′; 2		
1″/	6′; 3″	5′/	1′; 3′; 4′; 6′	6′/	1′; 2′; 2		
2″/	5′; 3″						

Table 3. ^{13}C NMR chemical shift for compounds **1**–**5** and selected carbons in the model compounds **6**–**8**

C	**1**	**2**	**6**	**7**	C	**3**	**4**	**5**	**8**
1	130.8	131.2	129.3	138.8	1′	135.0	134.2	126.9	120.2
2	105.3	105.7	111.5	129.2	2′	103.2	103.2	103.4	147.0
3	153.4	153.5	151.8	128.2	3′	153.5	153.4	153.6	145.7
4	139.5	141.0	149.9	127.7	4′	138.0	138.1	140.9	149.5
5	153.4	153.5	112.0	128.2	5′	153.5	153.4	153.6	147.6
6	105.3	105.7	112.9	129.2	6′	103.2	103.2	103.4	106.6
1′	105.9	105.8	106.9	106.0	10	105.9	105.9	112.9	112.5
2′	167.9	168.5	168.8*	155.4	5	164.9	164.8	154.5	154.7
3′	92.5	93.9	94.4	102.7	6	139.0	93.1	140.4	140.4
4′	161.3	166.3	166.5*	156.3	7	166.0	165.9	157.8	157.7
5′	102.9	91.5	91.6	102.5	8	96.4	93.5	96.3	96.2
6′	156.0	162.5	162.7	161.6	9	162.3	162.2	152.6	152.5
β	142.3	142.4	142.9	132.1	2	79.7	79.3	161.0	159.1
α	126.5	127.0	126.1	124.8	3	45.8	45.7	108.3	112.5
β′	192.4	192.4	188.0	193.0	4	189.2	189.0	177.2	177.2
					OMe	56.2		56.4	56.3
OMe	55.9	55.6	55.1		OMe	56.2	55.5	56.4	56.6
OMe	55.9	55.8	55.4		OMe	56.2	56.1	56.4	—
OMe	55.9	55.8	55.5		OMe	60.9	56.1	61.1	61.3
OMe	61.3	56.2	55.6		OMe	61.4	56.1	61.6	61.5
OMe		61.0			OMe	61.7	60.7	62.2	62.2

Assignments based on HMQC/HMBC for **1**, **2**, **4** and **5**. all in $CDCl_3$.
1: 1″ = 116.9; 2″ = 124.2; 3″ = 77.7; 4″/5″ = 28.1.
* Data obtained in this study suggest that these resonances were previously incorrectly assigned.

implies that the methoxyl in the A-ring is located at C-4′ (δ 55.9), and determined the position of the chromene ring between C-5′ and O-6′. This was supported by the ^1H NMR spectrum run in C_6D_6 which showed upfield shift ($\Delta\delta > 0.3$) for three methoxyls [8]. Moreover, the existence of correlation between the ^1H signal at δ 6.07, assigned to H-3′ (by HMQC and HMBC), and the ^{13}C signal at δ 102.9 (C-5′), confirmed the position of the chromene ring between the C-5′ and O-6′. The new natural product is therefore 2′-hydroxy-3,4,4′,5-tetramethoxy-5′,6′-(2″,2″-dimethyl-pyran)chalcone (**1**). The structural assignment was also supported by comparison of the ^{13}C NMR spectrum with those of 2′-hydroxy-3,4,4′,6′-tetramethoxychalcone (**6**) and 2′-hydroxy-3′,4′,5′,6′-di-(2″,2″-dimethylpyran)chalcone (**7**, flemiculosin) [9].

It has been noted that the H-β and C-β of a chalcone are more deshielded than the H-α and C-α [9]. However, HMQC experiments of **1** showed correlations of ^1H signals at δ 8.05 and 7.72 to the ^{13}C signals at δ 126.5 and 142.3, respectively, indicating apparent anomalies. The signal (δ 6.86) assigned to H-2 and H-6 showed one-bond correlation with the ^{13}C signals at δ 105.3. The methine proton at δ 7.72 showed cross peaks with the C-2/C-6 signal at δ 105.3 (^3J), so permitting the assignment of the signal at δ 7.72 to H-β.

Compound **2** gave spectral data in agreement with those published [6] for the 2′-hydroxy-3,4,4′,5,6′-pentamethoxychalcone which has previously been isolated from *Merrillia caloxylon* (Citroideae). However, the ^{13}C NMR data for **2** are reported here for the first time (Table 3). Compound **1** and **6** were used as a model. HMQC and HMBC (Table 2) experiments with **2** permitted the assignment of the signal at δ 7.71 to H-β, as in **1**, and suggested that the highly

oxygenated B-ring may have caused a upfield shift for H-β.

Compound **3** was isolated with a trace of 5,7,3′,4′,5′-pentamethoxyflavanone (**4**), previously isolated from *N. alba* [3]. The mixture was analysed by GC-mass spectrometry, which established that the flavanones were **4** and **3**. The presence of fragment ion at *m/z* 194 (100%) in the mass spectrum for **3**, associated with retro-Diels–Alder cleavage of C-ring, clearly indicated the presence of three methoxyls in the B-ring. Thus, a singlet at δ 6.68 in the ^{1}H NMR spectrum (Table 1) was assigned to H-2′ and H-6′, requiring the presence of methoxyls at C-3′, C-4′ and C-5′. The fragment ion at *m/z* 211, also associated with retro-Diels–Alder cleavage of C-ring, suggested that the A-ring carried three methoxyls. Therefore, three isomers are possible for this compound. The correct one was decided on the basis of ^{13}C NMR spectrum (Table 3) which showed signals for three methoxyl groups attached to *ortho*-disubstituted carbon (δ 60.9, 61.4 and 61.7). This implies that the methoxyls in the A-ring is located at C-5, C-6 and C-7, since the ^{1}H signal at δ 6.38 (1H) for unsubstituted methine indicate that this proton must not be vicinal to C=O (C-4). A comparison of ^{13}C chemical shifts of 5,7,8-trimethoxyflavanone (δ 89.5, C-6) 5,7,8- (δ 93.7, C-6) and 5,6,7-trimethoxyflavones (δ 96.7, C-8) indicated that C-8 methine resonance at lowerfield than C-6 methine [9]. Based on this evidence, the ^{13}C signal at δ 96.4 for C-8 supported the above proposed. The structure of the new natural product is therefore 5,6,7,3′,4′,5′-hexamethoxyflavanone (**3**).

Compound **5** gave spectral data in agreement with those published [7] for the 5,6,7,3′,4′,5′-hexamethoxyflavone which has previously been isolated from *Murraya paniculata* (Citroideae). However, the assignments of ^{13}C resonances for **5** are reported here for the first time (Table 3). Compound **3** and 5,6,7,2′,3′,4′,5′-heptamethoxyflavone (**8**) [9] were used as a models.

These results do not seem to support the affiliation of *Neoraputia* to the Cusparieae [4]. However, many chemists are perhaps interested in alkaloids, coumarins and limonoids from Rutaceae, and will rarely identify all of the potentially systematic important classes of compounds present in the plant, e.g. flavonoids, since they are generally found in most if not all angiosperm plants. Thus, clearly rutaceous flavonoids deserve more attention than they have received so far.

EXPERIMENTAL

General. NMR: on a Bruker ARX 400, with TMS as int. standard; GC-MS: low resolution on a HP-2576 instrument; IR (KBr, BOMEM-FtIR); UV (Perkin–Elmer).

Plant material. Neoraputia magnifica var. *magnifica* was collected in Espirito Santo, Brazil, and a voucher (SPF 81-316) is deposited in the Herbarium of Instituto de Ciências Biológicas-USP-São Paulo.

Isolation of compounds. Ground stems (850 g) was extracted with hexane, then CH_2Cl_2 and finally with MeOH. The conc. hexane extract was submitted to vacuum chromatography over silica gel using a hexane–CH_2Cl_2–MeOH gradient to give a mixt. of fatty acids followed by 3 frs. Fr. containing **1** and a mixt. of sterols was then flash rechromatographed as above and then by prep. TLC (silica gel, CH_2Cl_2–MeOH, 99:1) to yield 0.7 mg of pure **1** and a mixt. of sterols (2 mg). This mixt. was analysed by GC-MS, which established that the sterols were sitosterol and stigmasterol. Fr. 2 was purified by prep. TLC as above to give **2** (9 mg). Fr. 3 was subjected to reverse-phase chromatography on silanized silica gel with a gradient mixt. of H_2O–MeOH providing 10 frs. Fr. 4 was flash rechromatographed over silica gel using a hexane–EtOAc gradient, to give the following compounds: **3** (1.2 mg; with trace of **4**), **4** (1 mg) and **5** (1.5 mg).

2′-Hydroxy-3,4,4′,5-*tetramethoxy*-5′,6′-(2″,2″-*dimethylpyran*) *chalcone* (**1**). Yellow powder; UV λ_{max}^{MeOH} nm: 286, 352; IR ν_{max}^{KBr} cm^{-1}. 3348, 1620, 1549, 1503, 1455, 1247, 1129. ^{1}H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): see Table 3; HMQC (400/100 MHz, $CDCl_3$); HMBC (400/100 MHz, $CDCl_3$): see Table 2.

5,6,7,3′,4′,5′-*Hexamethoxyflavanone* (**3**, *with trace of* **4**). Yellow powder; UV λ_{max}^{MeOH} nm: 230, 280, 312; IR ν_{max}^{KBr} cm^{-1}: 1670, 1598, 1453, 1365, 1259. ^{1}H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$): see Table 3. GC-MS of **3**: (GC: HP-1 column 50 m × 0.25 mm programmed from 200–280° at 4.5° min^{-1}) R_t34.128 min. MS *m/z* (rel. int.): 404 $[M]^+$ (30), 237 (7), 211 (11), 210 (1), 195 (82), 194 (100), 192 (4), 179 (62), 151 (2).

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