

Further Pyrano Flavones From *Neoraputia alba*

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De *Neoraputia alba* (Rutaceae) foram isoladas oito flavonas, além de uma flavanona. Três das flavonas estão sendo descritas pela primeira vez: 3',4'-metilenodióxi-6,5-(2'',2''-dimetilpirano)-7-metóxi-flavona (1); 3',4',8-trimetóxi-5-hidróxi-6,7-(2'',2''-dimetilpirano)-flavona (2) e 3',4'-metilenodióxi-5,7,8-trimetóxi-flavona (3). Cinco substâncias conhecidas (4-8) foram também isoladas: 3',4'-metilenodióxi-5,5',6,7-tetrametóxi-flavona; 3',4',5,5',7-pentametóxi-flavona; 4',5,7,8-tetrametóxi-flavona; 3',4',5,7,8-pentametóxi-flavona; 3',4',5,5',7,8-hexametóxi-flavona. A única flavanona, 3',4',5,5',7-pentametóxi-flavanona (9), é conhecida como produto de derivatização, sendo esta a primeira vez que é isolada de plantas.

Neoraputia alba (Rutaceae) yielded eight flavones, besides one flavanone. Among the flavones, three were identified as the new compounds, 3',4'-methylenedioxy-6,5-(2'',2''-dimethylpyran)-7-methoxyflavone (1); 3',4',8-trimethoxy-5-hydroxy-6,7-(2'',2''-dimethylpyran)-flavone (2), 3',4'-methylenedioxy-5,7,8-trimethoxyflavone (3). Five known compounds (4-8) 3',4'-methylenedioxy-5,5',6,7-tetramethoxy-flavone; 3',4',5,5',7-pentamethoxyflavone; 4',5,7,8-tetramethoxy-flavone; 3',4',5,7,8-pentamethoxyflavone; 3',4',5,5',7,8-hexa-methoxyflavone were also isolated. The only flavanone (9), 3',4',5,5',7-pentamethoxyflavanone, is known as product of derivatization, however this is its first isolation from plants.

Key words - *Neoraputia alba*; *Rutaceae*, *flavones*.

Introduction

The genus *Raputia* was first described by Aublet in 1775, with the species *R. aromatica*. However, recently, Emericich¹ has renamed it as *Neoraputia*.

In a previous paper² we described the isolation of two pyrano flavones of *Neoraputia alba*. Now, we report the isolation and identification of eight flavones and one flavanone from the same plant.

Experimental

Extraction and isolation of the constituents - Leaves (1.041 kg) of *N. alba* (a voucher is deposited at the Herbarium of Instituto de Biociências-USP-São Paulo) collected in Espírito Santo, Brazil, were ground and extracted with petrol, CH₂Cl₂ and MeOH respectively. Solvent evaporation yielded the corresponding extracts. The petrol extract (2.082 g) afforded two flavonoids reported in a different paper². The methylene

chloride extract (14.527 g) was chromatographed on dry column (eluent CH₂Cl₂:EtOAc 9:1). The column was cut and the silica extracted with MeOH yielding 8 new fractions. Fraction 4 chromatographed over silicagel column yielded (3) (116 mg). Fractions 6,7 and 8 after recrystallization afforded (3) (86 mg), (4) (231 mg) and (8) (54 mg) respectively.

The methanol extract (18.470 g) was partitioned in MeOH/H₂O/hexane. The methanolic fraction partitioned with EtOAc/H₂O/H⁺. The acidic solution was neutralized with NH₄OH and extracted again with EtOAc. The resulting EtOAc extract after column chromatography yielded (3) (37 mg), (4) (118 mg) and (5) (121 mg).

From a second collect the dichloromethane extract of leaves (20.417 g) after a similar treatment as described above, yielded (1) (320 mg), (2) (13 mg), (6) (410 mg) and (7) (36 mg). Other extracts from stems of the same plant were also fractionated yielding the same compounds described above.

3',4'-Methylenedioxy-6,5-(2'',2''-dimethylpyran)-7-methoxyflavone (1). Colorless crystals from MeOH, m.p. (156-

158) °C. UV λ_{\max} nm: 308 (log ϵ 3.95), 330 (log ϵ 4.02). IR ν_{\max} cm⁻¹: 1645, 1515, 1473.

¹H NMR: table 1. ¹³C NMR: Table 3. MS *m/z* (rel. int. %): 378(16), 363(100), 217(83), 146(16), 145(14).

3',4',8-Trimethoxy-5-hydroxy-6,7-(2'',2''-dimethylpyran)-flavone (2). Yellowish crystals from MeOH, m.p. (182-184)°C. UV λ_{\max} nm: 294 (log ϵ 4.14), 350 (log ϵ 4.01). IR ν_{\max} cm⁻¹: 3435, 1656, 1620, 1575. ¹H NMR: table 1. ¹³C NMR: Table 3. MS *m/z* (rel. int. %): 410(76), 395(100), 165(34). Compound (2) was acetylated with acetic anhydride and pyridine yielding (2a).

¹H NMR: Table 1. **Conversion of (2) in (2b).** A mixture containing 1.4 mg of NaH, 10 mg of (2) and 1ml of CH₃I dissolved in mineral oil and DMF was stirred under N₂ for 40 min. Workup of the reaction yielded (2b) (7 mg). ¹H NMR: Table 1.

5,7,8-Trimethoxy-3',4'-methylenedioxyflavone (3). Colorless crystals from MeOH, m.p. (241-243)°C. UV λ_{\max} nm: 260 (log ϵ 4.13), 332 (log ϵ 4.14). IR ν_{\max} cm⁻¹: 1649, 1598, 1504, 1379. ¹H NMR: Table 1. ¹³C NMR: Table 3. MS *m/z* (rel. int. %): 356(35), 341(39), 146(61), 195(23), 167(100).

Results and discussion

The stems and leaves of *N. alba* yielded nine flavonoids, eight of them flavones and one flavanone.

Compounds 1 and 2 have very similar structures. The ¹H NMR for compound 1 (Table 1) showed features of a dimethylchromene ring (δ 5.56 and 6.58 vinylic protons), a singlet for a pair of magnetically equivalent methyl groups (δ

Table 1. ¹H NMR data for flavones 1, 2, 2a, 2b and 3 (CDCl₃, 200MHz, δ scale)

H	1	2	2a	2b	10	3
3	6.44s	6.54s	6.48s	6.60s	6.58s	6.52s
6						6.43s
8	6.43s					
2'	7.27d	7.37d	7.35d	7.43s	7.41d	7.33d
5'	6.87d	6.95d	6.93d	6.99d	6.98d	6.90d
6'	7.40dd	7.54dd	7.51dd	7.57dd	7.56dd	7.47dd
3''	5.56d	5.63d	5.85d	5.74d	5.66d	
4''	6.58d	6.69d	6.47d	6.74d	6.58d	
5'', 6''	1.51s	1.52s	1.52s	1.54s	1.54s	
OMe	3.89s	3.96s	3.97s	3.99s	4.05s	3.99s
OMe		3.95s	3.93s	3.98s	3.98s	3.96s
OMe		3.93s			3.97s	0.94s
OMe				3.89s	3.97s	
OCH ₂ O	6.02s					6.04s
CH ₃ CO			2.42s			

J(Hz): compounds 2, 2a, 2b, 3, 10: 2',6'=2; compound 1: 2',6'=1.8; compounds 2, 2a, 2b: 5',6'=8.5; compound 3: 5',6'=7.6; compound 1, 10: 5',6'=8; compounds 1, 2, 2a, 2b, 10: 3'',4''=10.

Table 2. *Eu(fod)*₃-induced ¹H NMR $\Delta\delta$ values*

H	1	2	10
3''	2.00	0.40	1.90
4''	1.26	0.90	1.06
5'',6''	3.20	0.40	2.80

* Shift studies were carried out by step wise addition of known amounts of *Eu(fod)*₃ to solutions of substrate in CDCl₃. The $\Delta\delta$ values were obtained by graphic extrapolation of observed shifts to 1:1 shift reagent substrate ratio.

1.51). Other signals referring to an ABX coupling system for three protons in the ring B (δ 7.40dd, 7.27d and 6.87d), a methoxyl (δ 3.89) and methylenedioxy (δ 6.02) were all well-defined in the ¹H NMR. From MS, the fragments *m/z* 149 and 146 indicated that the methylenedioxy is fused to the ring B, as well as the one *m/z* 217 requires the chromene fused to A. The comparison of these data to those reported for two other flavones isolated from the same plant², suggests that (1) contains one proton in the ring A, and a methylenedioxy in B. The relative positioning of the chromene ring came from experiments of lanthanide reagent³ (Table 2), which suggested the same arrangement as obtained for the two previously reported flavones². However, two possible isomers can be drawn for (1), which can either have a proton at C-7 and a methoxyl at C-8 or vice-versa. The decision for the compound containing 7-OMe and H-8 came from both biosynthetic arguments, where an oxygen atom is required in C-7 rather than C-8, and the chemical shift observed for C-8 (δ 91.4) in the ¹³C NMR, that would be a very high field for C-7⁴. The structure proposed is also in agreement with the ¹³C NMR (Table 3).

Compound 2 showed to be very closely related to (1). The ¹H NMR for this compound also showed features of a chromene ring, vinylic and methyl protons (Table 1). Again from ¹H NMR it was possible to assign a disubstituted B ring exhibiting a characteristic ABX coupling system. The presence of a chelated hydroxyl (δ 12.75) requires a hydroxyl group attached to C-5, as well as the chemical shift observed for C-4 (δ 182.3)⁴. Acetylation of (2) yielded the corresponding monoacetate (2a) (δ 2.42). The acetate (2a) also showed a deshielding for the signal of H-4'' ($\Delta\delta$ 0.21) displaying a linear arrangement for the chromene ring in this molecule. Compound (2) was also methylated yielding (2b), which showed to be an isomer of (10), a flavone described before², indicating a different attachment of the chromene unit to the ring A. Moreover, the addition of lanthanide shift reagent to (2a) in the ¹H NMR showed a large shift for the signals of H-3, H-4'' and H-3'' confirming that (2b) is an isomer of (10) (Table 3). Therefore a linear arrangement for the chromene ring was proposed for (2).

Compound (3) showed ¹H NMR signals referring to a methylenedioxy group (δ 6.04), three methoxyls (δ 3.99, 3.96 and 3.94). Further analysis from the ¹H NMR indicated the disubstituted pattern of the ring B, and the presence of one

Table 3. ^{13}C NMR data for flavones **1**, **2** and **3** (CDCl_3 , 50.32 MHz, δ scale).

C	1	2	3
2	160.2	163.1	160.3
3	107.9	108.4	106.1
4	177.1	182.3	177.6
5	154.2	151.5	156.3
6	106.0	105.7	93.1
7	158.6*	152.1	156.5
8	91.4	128.3	131.0
9	158.5*	149.1	151.9
10	108.6	104.7	107.5
1'	125.7	123.5	125.7
2'	106.4	110.9	108.7
3'	148.3	149.1*	148.5
4'	150.1	149.3*	151.9
5'	108.8	110.9	109.2
6'	120.8	119.8	121.1
2''	77.3	78.0	
3''	128.3	128.0	
4''	115.9	115.5	
5'', 6''	27.6	28.0	
OMe	55.9	61.4	61.6
OMe		55.9	56.7
OMe		55.7	56.4
OCH ₂ O	101.8		

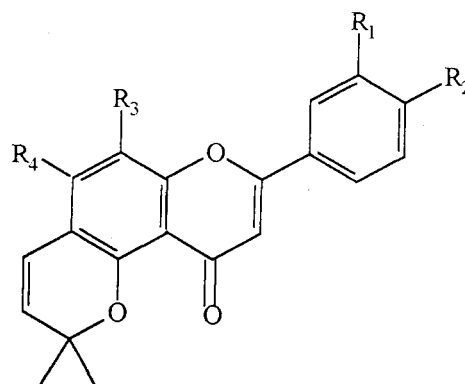
*Assignments may be interchangeable.

proton in the ring A (δ 6.43) as well. The suggestion that the methylenedioxy should be attached to the ring B, appeared from the mass spectrum m/z 146. One further problem was the location of the third methoxyl group in the ring A. This was decided on the basis of the ^{13}C NMR which showed only one signal for the methoxyl carbons (δ 61.58) requiring an *ortho*-disubstituted carbon⁵. This result conducted to the structure containing 8-OMe, instead of a 6-OMe.

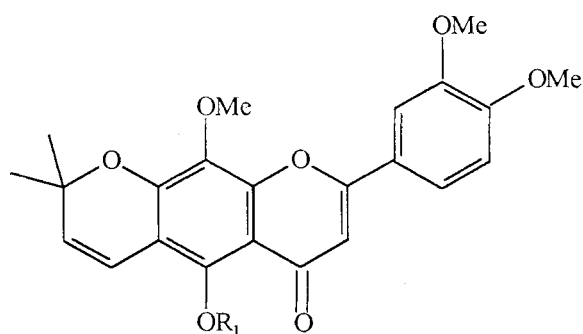
Flavones **4-8** were identified through comparison of their spectral data with the ones described in the literature⁶⁻⁸.

The use of C_6D_6 was necessary to decide among some possible isomers, especially for those compounds having three methoxyls in the ring A. It has been shown that C_6D_6 induces a shielding in methoxyl signals, in the ^1H NMR, when this group has an *ortho* hydrogen^{9,10}. Using this experiment together with the chemical shifts observed for the methoxyl carbons in the ^{13}C NMR, it was possible to distinguish among the isomers for compounds **4**, **6**, **7** and **8**. For example, when the ^1H NMR was run for compound **8** in CDCl_3 and subsequently in C_6D_6 , a shielding was observed in the signals for four methoxyls. This means that only two methoxyls have no proton in *ortho* positions. This result led unequivocally to the structure **8** for this flavone. A similar approach was used for the other compounds.

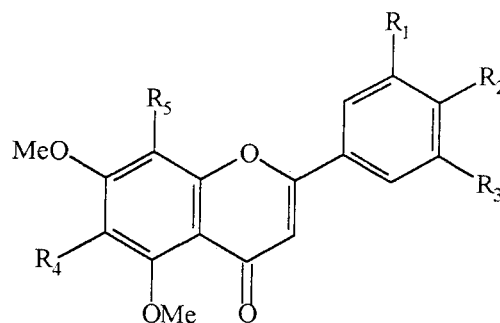
The only flavanone isolated from this plant was identified on the basis of its spectral data and comparison with those reported in the literature for the same compound¹¹.



1 $\text{R}^1 = \text{R}^2 = \text{OCH}_2\text{O}$, $\text{R}^3 = \text{H}$, $\text{R}^4 = \text{OMe}$
10 $\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{R}^4 = \text{OMe}$



2 $\text{R}_1 = \text{H}$
2a $\text{R}_1 = \text{Ac}$
2b $\text{R}_1 = \text{Me}$



3 $\text{R}^1 = \text{R}^2 = \text{OCH}_2\text{O}$, $\text{R}^3 = \text{R}^4 = \text{H}$, $\text{R}^5 = \text{OMe}$
4 $\text{R}^1 = \text{R}^2 = \text{OCH}_2\text{O}$, $\text{R}^3 = \text{R}^4 = \text{OMe}$, $\text{R}^5 = \text{H}$
5 $\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{OMe}$, $\text{R}^4 = \text{R}^5 = \text{H}$
6 $\text{R}^1 = \text{R}^3 = \text{R}^4 = \text{H}$, $\text{R}^2 = \text{R}^5 = \text{OMe}$
7 $\text{R}^1 = \text{R}^2 = \text{R}^5 = \text{OMe}$, $\text{R}^3 = \text{R}^4 = \text{H}$
8 $\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{R}^5 = \text{OMe}$, $\text{R}^4 = \text{H}$
9 $\text{R}^1 = \text{R}^2 = \text{R}^3 = \text{OMe}$, $\text{R}^4 = \text{R}^5 = \text{H}$. 2. 3-dihydro

The occurrence of polymethoxylated flavones is important for the chemosystematics of *Neoraputia*. This is the second time that this type of compounds has been isolated from this genus. Polymethoxylated flavones have also been found in *Citrus* species^{12,13}

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