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## Flavones from the Leaves of *Ficus gomelleira*

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Duas novas flavonas, 5-hidroxi-7,5'-dimetoxi-3',4'-metilenodioxiflavona e 5-hidroxi-7,3',5'-trimetoxi-4'-(3,3-dimetilaliloxi)flavona foram isoladas das folhas de *Ficus gomelleira*, juntamente com três flavonas conhecidas, a 5,6,7,3',4',5'-hexametoxyflavona, 5-hidroxi-8,3',4'-trimetoxi-2'',2''-dimetilpirano (5'',6'':6,7)-flavona e 5-hidroxi-8,3',4',5'-tetrametoxi-2'',2''-dimetilpirano (5'',6'':6,7)-flavona. As estruturas das substâncias isoladas foram elucidadas com base na análise de seus dados espectrais, bem como por comparação com dados da literatura.

Two new flavones, 5-hydroxy-7,5'-dimethoxy-3',4'-methylenedioxyflavone and 5-hydroxy-7,3',5'-trimethoxy-4'-(3,3-dimethylallyloxy)flavone, as well as three known flavones: 5,6,7,3',4',5'-hexamethoxyflavone, 5-hydroxy-8,3',4'-trimethoxy-2'',2''-dimethylpyrano (5'',6'':6,7)-flavone and 5-hydroxy-8,3',4',5'-tetramethoxy-2'',2''-dimethylpyrano (5'',6'':6,7)-flavone were isolated from the leaves of *Ficus gomelleira*. Their structures were elucidated by spectroscopic methods and comparison with literature data.

**Keywords:** *Ficus gomelleira*, Moraceae, leaves, flavones

## Introduction

In the course of our systematic studies on the species of genus *Ficus* of medicinal interest, which grows in the Amazon forest, we have investigated *F. gomelleira*. The leaves and latex of this species are used in folk medicine for intestinal diseases as laxative and vermifuge<sup>1</sup>. Earlier studies on the genus *Ficus* (*F. maxima* and *F. aripuanensis*), carried out in our laboratory, have resulted in the isolation of flavonoids and terpenoids<sup>2,3</sup>. From *F. gomelleira* two novel flavones, 5-hydroxy-7,5'-dimethoxy-3',4'-methylenedioxyflavone (**1**) and 5-hydroxy-7,3',5'-trimethoxy-4'-(3,3-dimethylallyloxy)flavone (**2**) were obtained, and the known flavones 5,6,7,3',4',5'-hexamethoxyflavone (**3**)<sup>4</sup>, 5-hydroxy-8,3',4'-trimethoxy-2'',2''-dimethylpyrano (5'',6'':6,7)-flavone (**4**)<sup>5</sup> and 5-hydroxy-8,3',4',5'-tetramethoxy-2'',2''-dimethylpyrano (5'',6'':6,7)-flavone (**5**)<sup>6</sup>. In this paper we describe the isolation and structural elucidation of **1** and **2**.

## Experimental

### General experimental procedures

IR spectra were taken on a Perkin Elmer 281 Spectrometer. <sup>1</sup>H NMR (300 and 500 MHz) and <sup>13</sup>C NMR (75 and 125

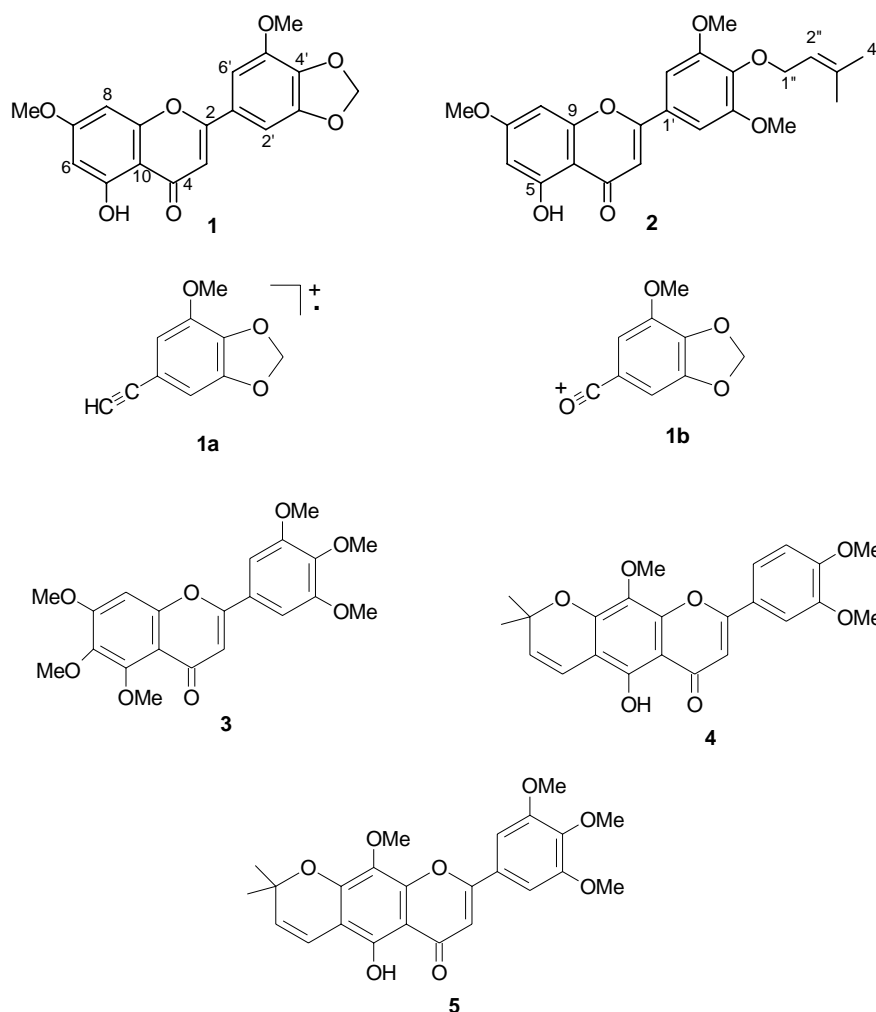
MHz) spectra were recorded on a Varian GEMINI 300 and a Bruker DRX 500, in CDCl<sub>3</sub>, with residual solvent peak as int. reference. The MS runs were performed using a Micromass Platform II instrument (Micromass, Wythenshawe, Manchester, UK) equipped with an EI/CI ion source. Electrospray and CAD data were collected in a triple quadrupole Micromass Quatro LC instrument (Micromass, Wythenshawe, Manchester, UK) equipped with a "Z-spray" ion source. Chromatography was performed using silica gel (Merck) for column and preparative TLC. The purity of the compounds was checked by <sup>1</sup>H and <sup>13</sup>C NMR spectral analysis and TLC plate, revealed with UV (254 nm) and ceric sulphate (0.2 %) in H<sub>2</sub>SO<sub>4</sub> (5 %).

### Plant material

Leaves of *F. gomelleira* were collected in Marituba County, State of Pará, Brazil. Voucher specimen n° 0147615 has been deposited at the Herbarium of the Emílio Goeldi Museum, Department of Botany, Belém, Pará, Brazil.

### Extraction and isolation of flavones

The ground dried leaves (3.0 kg) were extracted at room temperature with hexane and CH<sub>2</sub>Cl<sub>2</sub>, successively. The hexane extract (11.0 g) was chromatographed on silica gel and eluted



with a gradient of acetone in hexane to give 20 fractions. Fr. 10 eluted with hexane-acetone (9:1) was purified by repeated column chromatography on silica gel with hexane-CH<sub>2</sub>Cl<sub>2</sub>-acetone mixtures of increasing polarity, followed by prep. TLC (silica gel, hexane-EtOAc 9:1, 5 runs) to give **2** (3 mg). Fr. 17 eluted with hexane-acetone (8:2) was submitted to repeated CC on silica gel with of hexane-CHCl<sub>3</sub>-EtOAc mixtures, followed by prep. TLC (silica gel, developed with CHCl<sub>3</sub>, 3 runs) to yield **3** (10 mg).

The dichloromethane extract (29.0 g) was subjected to CC on silica gel and eluted with hexane-CH<sub>2</sub>Cl<sub>2</sub>-MeOH mixtures of increasing polarity affording 47 fractions. Fr. 22 eluted with hexane-CH<sub>2</sub>Cl<sub>2</sub> (6:4) was purified twice by CC on silica gel with mixtures of hexane-CHCl<sub>3</sub>-MeOH affording **1** (9 mg). Fr. 28 eluted with hexane-CH<sub>2</sub>Cl<sub>2</sub> (1:1) was separated by repeated CC on silica gel with mixtures of hexane-CH<sub>2</sub>Cl<sub>2</sub>-acetone of increasing polarity affording **5** (16 mg). Fr. 29 eluted with hexane-CH<sub>2</sub>Cl<sub>2</sub> (4:6) was purified after repeated CC on silica gel with mixtures of hexane-CHCl<sub>3</sub>-acetone, followed by prep. TLC (silica gel, hexane-EtOAc 7:3, 3 runs) to yield **4** (6 mg). The identification of the known

compounds **3-5** involved the analysis of <sup>1</sup>H and <sup>13</sup>C NMR spectral data and comparison with literature values<sup>4-6</sup>.

*5-hydroxy-7,5'-dimethoxy-3',4'-methylenedioxyflavone (1)*. Brown oil. UV  $\lambda_{\max}/\text{nm}$  (MeOH) (log  $\epsilon$ ): 260 (3.32), 332 (3.09), 380 sh (2.98); UV  $\lambda_{\max}/\text{nm}$  (MeOH + AlCl<sub>3</sub>) (log  $\epsilon$ ): 257 (3.50), 340 (3.06), 380 sh (2.98); UV  $\lambda_{\max}/\text{nm}$  (MeOH + AlCl<sub>3</sub> + HCl) (log  $\epsilon$ ): 254 (3.60), 341 (3.09), 381 sh (2.99). IR  $\nu_{\max}/\text{cm}^{-1}$  3405, 1655, 1601, 1491, 1447, 1164, 1045 (film). <sup>1</sup>H and <sup>13</sup>C NMR see Table 1; EIMS,  $m/z$  342 [M<sup>+</sup>] (100), 327 [M-CH<sub>3</sub>]<sup>+</sup> (3), 314 [M-CO]<sup>+</sup> (8), 312 (13), 299 (13), 179 (5), 176 (19), 166 (5).

*5-hydroxy-7,3',5'-trimethoxy-4'-(3,3-dimethylallyloxy)flavone (2)*. Brown oil. UV  $\lambda_{\max}/\text{nm}$  (MeOH) (log  $\epsilon$ ): 245 (3.97), 333 (3.98), 380 sh (3.43); UV  $\lambda_{\max}/\text{nm}$  (MeOH + AlCl<sub>3</sub>) (log  $\epsilon$ ): 277 (4.09), 348 (3.98), 385 sh (3.89); UV  $\lambda_{\max}/\text{nm}$  (MeOH + AlCl<sub>3</sub> + HCl) (log  $\epsilon$ ): 275 (4.23), 345 (3.97), 380 sh (3.87); IR  $\nu_{\max}/\text{cm}^{-1}$  3410, 2920, 1648, 1605, 1488, 1447, 1340, 1251, 1116 (film); <sup>1</sup>H and <sup>13</sup>C NMR see Table 1; ESIMS,  $m/z$  413 [M+H]<sup>+</sup> (29), 385 [M+H-CO]<sup>+</sup> (7), 371 (28), 345 [M+H-C<sub>5</sub>H<sub>8</sub>]<sup>+</sup> (100), 329 (29), 301 (30), 298 (9), 171 (14).

**Table 1.**  $^1\text{H}$  (300 MHz) and  $^{13}\text{C}$  (75 MHz) NMR spectral data for **1** and **2** in  $\text{CDCl}_3$ . Chemical shifts in  $\delta$  ( $\delta_{\text{H}}$  and  $\delta_{\text{C}}$ , ppm) and coupling constants ( $J$ , in parentheses) in Hz.\*

	<b>1</b>		<b>2</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
<b>C</b>				
2	163.5		163.9	
4	182.4		182.3	
5	162.2		162.3	
7	165.6		165.6	
9	157.7		157.6	
10	105.2		105.6	
1'	125.7		126.4	
3'	149.6		154.1	
4'	138.6		140.6	
5'	143.9		154.1	
3''			139.4	
<b>CH</b>				
3	105.2	6.54 (s)	105.6	6.60 (s)
6	98.2	6.37 (d, 2.3)	98.2	6.38 (d, 2.1)
8	92.7	6.48 (d, 2.3)	92.7	6.50 (d, 2.1)
2'	107.3	7.06 (d, 1.4)	103.8	7.07 (s)
6'	100.7	7.09 (d, 1.4)	103.8	7.07 (s)
2''			120.3	5.56 (t, 7.2)
<b>CH<sub>2</sub></b>				
1''			69.7	4.58 (d, 7.2)
OCH <sub>2</sub> O	102.3	6.08 (s)		
<b>CH<sub>3</sub></b>				
MeO-7	56.82	3.88 (s)	55.8	3.84 (s)
MeO-3'			56.4	3.94 (s)
MeO-5'	56.97	3.99 (s)	56.4	3.94 (s)
4''			25.8	1.75 (s)
5''			17.9	1.68 (s)
HO-5		12.70 (s)		12.74 (s)

\*  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts were assigned from HETCOR [ $^{13}\text{C}\times^1\text{H}$ -COSY- $^1J(\text{CH})$ ], HMBC [ $^1\text{H}\times^{13}\text{C}$ -COSY- $^{2,3}J(\text{CH})$ ] and  $^1\text{H}\times^1\text{H}$ -NOESY spectra.

## Results and Discussion

Compound **1** was isolated as a brown oil and its molecular formula determined as  $\text{C}_{18}\text{H}_{14}\text{O}_7$  from NMR and mass spectral data. The UV spectral data indicated that it was a 5-hydroxyflavone<sup>7</sup>. The chelated hydroxyl hydrogen at  $\delta_{\text{H}}$  12.70 and the carbonyl group at  $\delta_{\text{C}}$  182.4 were also consistent with a 5-hydroxyflavone<sup>7,8</sup>. The aromatic region of the  $^1\text{H}$  NMR spectrum (Table 1) displayed five hydrogen resonance signals [ $\delta_{\text{H}}$  7.06 and 7.09 (d each,  $J$  1.4 Hz), 6.37 and 6.48 (d each,  $J$  2.3 Hz), and 6.54 (s)] which constitute two AB spin systems and an isolated hydrogen. These spectral data were in agreement with a 5,7,3',4',5'-substituted flavone<sup>7</sup>. In addition to a methylenedioxy signal at  $\delta_{\text{H}}$  6.08, two aromatic methoxyl resonances were observed at  $\delta_{\text{H}}$  3.88 and 3.99. The fragment peaks at  $m/z$  176 (**1a**) from a retro-Diels-Alder reaction in the EIMS and  $m/z$  179 (**1b**) from a retro-Diels-Alder reaction, followed by a rearrangement, were consistent with the ring B substituted with methylenedioxy and methoxy groups at 3',4' and 5' positions, respectively. As a consequence the ring A was substituted with the remaining methoxyl at C-7 and **1** was determined as 5-hydroxy-7,5'-dimethoxy-3',4'-methylenedioxyflavone. The proposed structure of **1** was further confirmed by  $^{13}\text{C}$  NMR data (Table 1). The correct

chemical shifts assignment of all hydrogen ( $\delta_{\text{H}}$ ) and carbon ( $\delta_{\text{C}}$ ) atoms of **1** was obtained using a combination of HETCOR ( $^{13}\text{C}\times^1\text{H}$ -COSY,  $^1J(\text{CH})$ ; Table 1), HMBC ( $^1\text{H}\times^{13}\text{C}$ -COSY,  $^{2,3}J(\text{CH})$ ; Figure 1) and NOESY ( $^1\text{H}\times^1\text{H}$  nOe dipolar interaction; Figure 1) experiments. Therefore, for example, the spin-spin interactions of OH-5 ( $\delta_{\text{H}}$  12.7) with C-5 ( $\delta_{\text{C}}$  162.2,  $^2J(\text{CH})$ ), C-6 ( $\delta_{\text{C}}$  98.2,  $^3J(\text{CH})$ ) and C-10 ( $\delta_{\text{C}}$  105.2,  $^3J(\text{CH})$ ), observed in the HMBC spectrum (Figure 1), have allowed the unequivocal assignment of  $\delta_{\text{C}}$  for C-5, C-6 and C-10. Based on the attribution of C-6 along with a heteronuclear direct one bond coupling between C-6 ( $\delta_{\text{C}}$  98.2) and H-6 ( $\delta_{\text{H}}$  6.37) revealed by the HETCOR spectrum, it was possible to establish the unequivocal chemical shift of H-6. As a consequence, the other *meta*-coupled hydrogen H-8 was attributed at  $\delta_{\text{H}}$  6.48. The OMe group at position 7 was assigned at  $\delta_{\text{H}}$  3.88, based on the  $^1\text{H}\times^1\text{H}$ -NOESY spectrum (Figure 1) which showed the dipolar interactions between  $\text{CH}_3\text{O}-7$  ( $\delta_{\text{H}}$  3.88) and both H-6 ( $\delta_{\text{H}}$  6.37) and H-8 ( $\delta_{\text{H}}$  6.48), and by cross peak of  $\text{CH}_3\text{O}-7$  ( $\delta_{\text{H}}$  3.88) and H-8 ( $\delta_{\text{H}}$  6.48) with C-7 ( $\delta_{\text{C}}$  165.6,  $^{2,3}J(\text{CH})$ ), exhibited in the HMBC spectrum. H-2' and H-6' showed doublets at  $\delta_{\text{H}}$  7.06 and 7.09, respectively. Their assignments were accomplished by the analysis of the NOESY spectrum, which showed a dipolar interaction between  $\text{CH}_3\text{O}-5'$  ( $\delta_{\text{H}}$  3.99) and H-6' ( $\delta_{\text{H}}$  7.09).

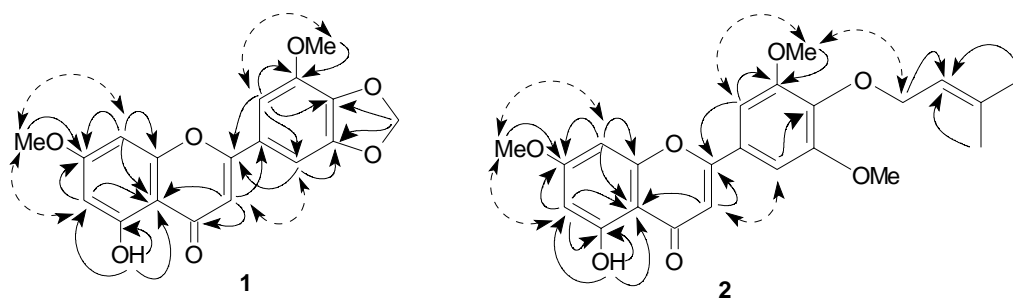


Figure 1. Observed CH long-range correlations in the HMBC ( $\rightarrow$ ) and  $^1\text{H} \times ^1\text{H}$ -NOESY ( $\leftrightarrow$ ) spectra for **1** and **2**, (500 MHz,  $\text{CDCl}_3$ )

The carbon C-4' is between two oxygenated positions, so it was attributed to the signal at  $\delta_{\text{C}}$  138.6. The  $\delta$  of the other oxygenated carbon atoms C-3' and C-5' were confirmed by the analysis of the HMBC spectrum, which revealed the cross peaks of  $\text{OCH}_2\text{O}$  ( $\delta_{\text{H}}$  6.08) with C-3' ( $\delta_{\text{C}}$  149.6,  $^3J(\text{CH})$ ) and between H-6' ( $\delta_{\text{H}}$  7.09) and  $\text{CH}_3\text{O}$ -5' ( $\delta_{\text{H}}$  3.99) with C-5' ( $\delta_{\text{C}}$  143.9,  $^2,3J(\text{CH})$ ). These data are also in agreement with those reported for the model compounds 5,4'-dihydroxy-7-methoxyflavone<sup>8</sup> and 5,6,7,8,5'-pentamethoxy-3',4'-methylenedioxy-flavone<sup>9</sup>.

Compound **2** was isolated as a brown oil and its molecular formula was determined as  $\text{C}_{23}\text{H}_{24}\text{O}_7$  from the NMR and electrospray ionization (ESI) mass spectra. The UV spectral pattern, including applications of shift reagents (see Experimental) and the chelated hydroxyl proton signal at  $\delta_{\text{H}}$  12.74 indicated that **2** was also a 5-hydroxyflavone<sup>7</sup>. The  $^1\text{H}$  NMR (Table 1) of this compound exhibited signals for three aromatic methoxy groups at  $\delta_{\text{H}}$  3.84 (3H) and 3.94 (6H), one prenyloxy moiety [ $\delta_{\text{H}}$  4.58 (d,  $J$  7.2 Hz, 2H-1'', 5.56 (t,  $J$  7.2 Hz, H-2'', 1.75 (s, 3H-4'') and 1.68 (s, 3H-5'')] and showed a substitution pattern of ring A identical to compound **1**, with two meta-coupled doublets (each  $J$  2.1 Hz) at  $\delta_{\text{H}}$  6.38 and 6.50, assigned to H-6 and H-8, respectively. The presence of a singlet at  $\delta_{\text{H}}$  7.07 (2H) assigned to H-2'/6' clearly indicated that ring B was symmetrically substituted with methoxyl groups at 3' and 5' positions. The  $^{13}\text{C}$  NMR spectrum (Table 1) showed signals for methoxyl groups at  $\delta_{\text{C}}$  55.8 and 56.4 indicating the absence of methoxyl groups ortho-disubstituted<sup>8</sup>, which implied that the remaining methoxyl was located at C-7 and the prenyloxy group, at C-4'. This was supported by NOE interactions between  $\text{OMe}$ -7 ( $\delta_{\text{H}}$  3.84) and both H-6 ( $\delta_{\text{H}}$  6.38) and H-8 ( $\delta_{\text{H}}$  6.50), as well as between 2H-1'' ( $\delta_{\text{H}}$  4.58) and  $\text{OMe}$ -3'/ $\text{OMe}$ -5' ( $\delta_{\text{H}}$  3.94) observed in the  $^1\text{H} \times ^1\text{H}$ -NOESY spectrum (Figure 1). The above data led us to formulate the new flavone **2** as 5-hydroxy-7,3',5'-trimethoxy-4'-(3,3-dimethylallyloxy)flavone. The proposed structure **2** was also in agreement with the  $^{13}\text{C}$  NMR data (Table 1), which were fully assigned using a combination of HETCOR,

HMBC and NOESY experiments, as summarized in Table 1 and Figure 1, similarly to what was done with the  $^{13}\text{C}$  NMR data of **1**. These results were also coherent with the data described in the literature for the model compounds 5,4'-dihydroxy-7-methoxyflavone<sup>8</sup> and 5,6,7,3',5'-pentamethoxy-4'-(3,3-dimethylallyloxy)flavone<sup>2</sup>.

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