

**<sup>1</sup>H and <sup>13</sup>C-NMR Assignments of Two New Derivatives from  
Lanaraflavone, a 4'-O-8'' Biflavone Dimer from *Ouratea hexasperma*  
(Ochnaceae)**

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**Keywords:** lanaraflavone, <sup>1</sup>H and <sup>13</sup>C NMR data, *biflavones*, *Ouratea hexasperma*; Ochnaceae.

**Abstract:** The 1D and 2D NMR techniques were used to make the complete <sup>1</sup>H and <sup>13</sup>C chemical shifts assignments of two new lanaraflavone derivatives, 5-hydroxy-7-methoxyflavone-(4'-O→8'')-5'',4'''-dihydroxy-7''-methoxyflavone, isolated from the leaves of *Ouratea hexasperma*, its acethyl derivative, 5-acethoxy-7-methoxyflavone-(4'-O→8'')-5'',4'''-diacethoxy-7''-methoxyflavone and the <sup>13</sup>C NMR data of lanaraflavone penta-methyl ether.

**Resumo:** O uso de técnicas de RMN 1D e 2D permitiu o completo assinalamento dos deslocamentos químicos de <sup>1</sup>H e <sup>13</sup>C de dois novos derivados da lanaraflavona, 5-hidroxi-7-metoxiflavona-(4'-O→8'')-5'',4'''-diidroxi-7''-metoxiflavona, isolado das folhas de *Ouratea hexasperma*, do derivado acetilado 5-acetoxi-7-metoxi-(4'-O→8'')-5'',4'''-diacetoxi-7''-metoxiflavona e os dados de RMN <sup>13</sup>C do penta-metil eter da lanaraflavona.

## Introduction

We have described the phytochemical<sup>1-5</sup> and pharmacological<sup>6-8</sup> study of *Ochnaceae* species including the NMR spectra data analysis of new derivatives of the biflavonoids that have been frequently found in this family. Continuing the investigations on *Ouratea* species, we have isolated a reasonable amount of 7,7''-dimethyl-lanaraflavone from *Ouratea hexasperma*, collected in the Mata Atlântica region in Paraíba state, Brazil<sup>5</sup>. A new prepared

lanaraflavone derivative permitted to confirm the proposed structure of natural dimethyl ether and to make the complete proton and carbon-13 chemical shifts assignment of both new biflavone derivatives (Figure 1).

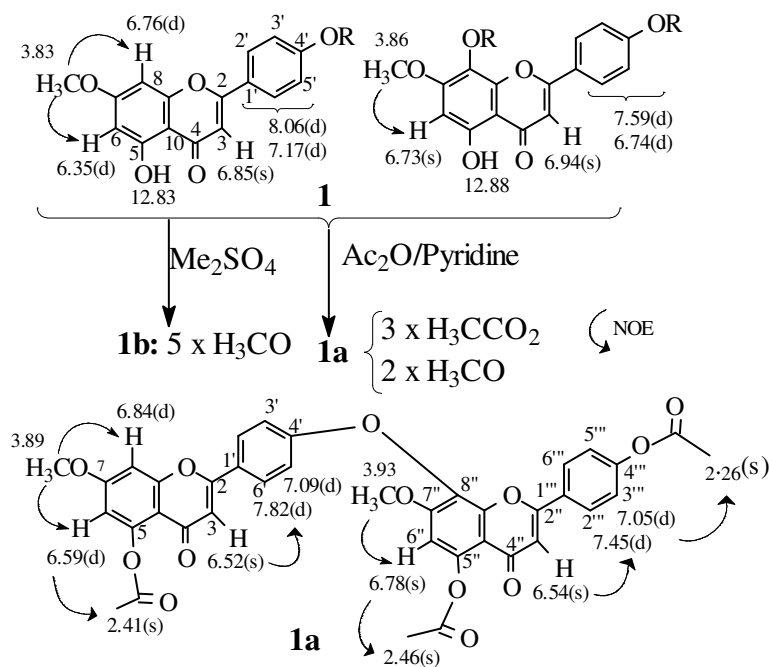
## Experimental

The leaves of *Ouratea hexasperma* St.-Hil were collected in João Pessoa, State of Paraíba, Brazil. A voucher specimen (N<sup>o</sup> JPB-21438) was deposited at the Herbarium

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Prof. Lauro Pires Xavier Universidade Federal da Paraíba, João Pessoa-PB, Brazil. Air dried leaves (585.0 g) were extracted exhaustively with  $\text{CH}_2\text{Cl}_2$  and MeOH at room temperature. The solvents were removed under vacuum to obtain the **LD** (14.52g) and **LM** (133.0 g) residues. The **LD** residue (13.5

g) was filtered on a silica gel column using  $\text{CH}_2\text{Cl}_2$ , AcOEt and MeOH. The residue from the fraction eluted with AcOEt (**LDA**, 4.0 g) was crystallized from MeOH to afford 7,7"-dimethyl-lanaraflavone (**1**, mp 325-328 °C, 98.2 mg).



**Figure 1.** Structures of lanaraflavone derivatives and the NOE detected by NOEDIFF experiments.

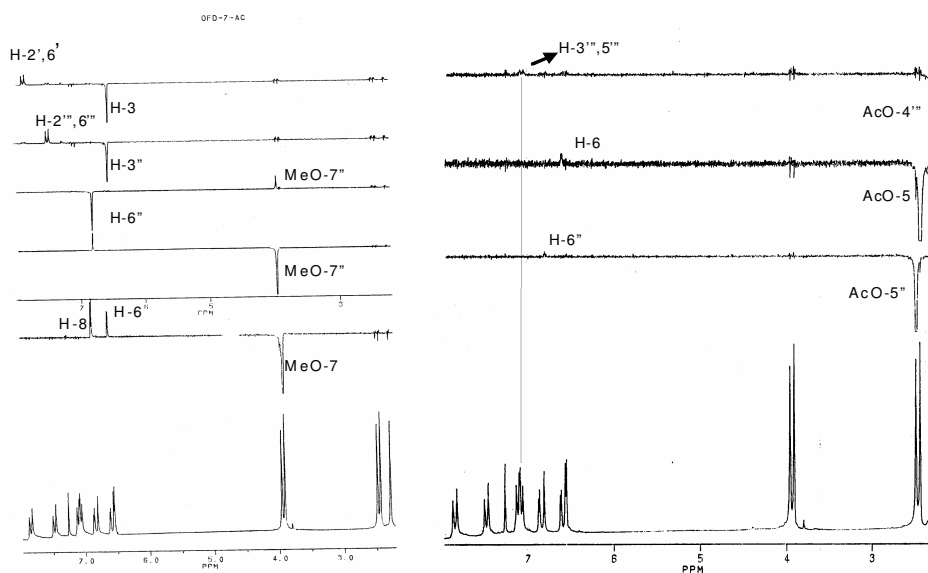
Compound **1** (33.0 mg, Figure 1) was dissolved in a mixture of pyridine (2.0 mL) and  $\text{Ac}_2\text{O}$  (2.0 mL). The solution was allowed to stand for 24h at room temperature. The usual work-up afforded a residue which was crystallized from MeOH to give the triacetyl derivative **1a** (mp 125-130°C, 20.6 mg, Figure 1). Treatment of **1** (50.0 mg) with dimethylsulfate yielded the penta-methyl derivative (**1b**, mp 265 °C, Figure 1). NMR spectra, in  $\text{D}_3\text{CSOCD}_3$  and  $\text{CDCl}_3$  solution in 5 mm tubes, were

recorded on Bruker ( $^1\text{H}$ : 200 MHz,  $^{13}\text{C}$ : 50.3 MHz) and on Jeol JNM-GX-400 ( $^1\text{H}$ : 400 MHz,  $^{13}\text{C}$ : 100 MHz) spectrometers using TMS as internal standard. Homonuclear 2D  $^1\text{H}\times^1\text{H}$ -COSY and heteronuclear 2D  $^1\text{H}\times^{13}\text{C}$ -COSY- $^n\text{J}_{\text{CH}}[n=1, \text{HMQC (modulated with } ^1\text{J}_{\text{CH}} = 140 \text{ Hz); } n=2 \text{ and } 3, \text{HMBC (modulated with } ^n\text{J}_{\text{CH}} = 9.0 \text{ Hz)]}$  spectra were obtained with Jeol pulse sequences. NOE-difference spectra were obtained using NOEDIFF.AU Bruker Ac-200 program.

## Results and Discussion

The  $^1\text{H}$  and  $^{13}\text{C}$  (BBD and DEPT) NMR spectral analysis of **1** was used to make the partial expanded molecular formula  $(\text{C}=\text{O})_2\text{C}_{15}(\text{CH})_{13}(\text{OMe})_2(\text{OH})_3$ . The chemical shift value of the quaternary carbons and the  $m/z$  566 (2%,  $\text{M}^+$ ) observed in the LREIMS indicated the molecular formula  $\text{C}_{32}\text{H}_{22}\text{O}_{10}$ . This formula is consistent with a biflavonyl ether skeleton with one free hydroxyl group ( $\delta_{\text{H}}$  10.9), two H-bonded hydroxyl groups ( $\delta_{\text{H}}$  12.83 and 12.88), two methoxyl groups ( $\delta_{\text{H}}$  3.83 and 3.86, s, 3H), four doublets at  $\delta_{\text{H}}$  8.06, 7.59, 7.17 and 6.74, corresponding to two AA'BB' systems in two *para*-substituted aromatic rings, three singlets at  $\delta_{\text{H}}$  6.94, 6.85 and 6.73 and two doublets (2.0 Hz) at 6.35 and 6.76. The  $\delta_{\text{CH}}$  at 104.8, 104.2, 98.1, 92.8 and 96.4 are compatible with C-3, -3'', -6, -8 and -6'', respectively, of **1**. Comparison of the

carbon chemical shifts of **1** with those of 7-methylflavanone found in the literature<sup>2</sup> revealed the shielding of the methyne carbons CH-6 (98.1), CH-8 (92.8), CH-6'' (96.4) through a  $\gamma$  effect of the carbon atom in the O-methyl group at C-7 and C-7''. The absence of an additional signal of CH near 93.0 ppm indicated that C-8'' was involved in the connection of the flavonoid moieties. The  $^1\text{H}\{^1\text{H}\}$ -NOE difference spectra of **1** with irradiation at 3.86 showed NOE at  $\delta_{\text{H}}$  6.73(s, H-6'') and irradiation at 3.83 showed NOE at 6.76 (d, 2 Hz, H-8) and 6.35 (d, 2.0 Hz, H-6) (Figure 2). This information along with the absence of a NOE on the doublets for H-3',5'' and 3'',5'' confirmed the location of the methoxy group at 7 and 7'', the chemical shift of H-6, H-8 and H-6'', and also the proposal of 4'-O $\rightarrow$ 8'' connection.



**Figure 2.**  $^1\text{H}$  NMR spectra (200 MHz) of **1a** from NOEDIFF experiments. Negative phase are from irradiated nuclei; signals from NOE are with positive phase.

The homonuclear 2D  $^1\text{H}\times^1\text{H}$ -COSY was used to identify the two AA',BB' systems of two *para* substituted aromatic rings. HMQC experiments showed  $^1J_{\text{CH}}$  of the hydrogen at  $\delta_{\text{H}}$  6.76, 6.73, 6.35, 6.94, 6.85, 7.17 and 8.06 with the respectively methyne carbon at  $\delta_{\text{CH}}$  92.8, 96.4, 98.01, 104.2, 104.8, 115.69, and 128.3. The same spectrum shows the cross peaks of the methyl groups at 3.87(MeO-7) and 3.91 (MeO-7'') with carbon signal at 56.1 and 56.9. The HMBC spectrum of **1** showed heteronuclear long-range ( $^2,^3J_{\text{CH}}$ ) couplings of MeO-7 and MeO-7'' with C-7 (165.3) and C-7'' (157.3), respectively; between H-6 (6.35)

with 104.2(C-10), 165.3 (C-7); HO-5 with C-5 (161.4),C-6(98.1) and with C-10(104.2); H-3 with C-2 (163.3) and C-4 (182.0); H-3',5' with C-4' (161.4), C-1'(124.7); HO-5'' with C-5'' (157.9), C-6'' (96.4) and with C-10'' (103.0); H-6'' with C-5'' (157.9), C-8'' (120.7) and C-7'' (157.3); H-3'' with C-2'' (163.8); H-3''',5''' with C-4'''(161.3), C-1'''(121.7); H-2''',6''' with C-4''' (161.3) and C-2'' (163.8); H-2',6' with C-4' (161.4) and C-2 (163.3). These data allowed us to make the complete chemical shift assignment of the quaternary carbons of **1** (Table 1).

**Table 1.**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (100 MHz) NMR spectral data of **1** in  $\text{D}_3\text{CSOCD}_3$ . Chemical shifts are in  $\delta$  (ppm) and coupling constants (J) in Hz.

	HMQC ( $^1J_{\text{CH}}$ )		HMBC	
	$\delta_{\text{CH}}$	$\delta_{\text{H}}$ (mult,Hz)	( $^2J_{\text{H-C}}$ )	( $^3J_{\text{H-C}}$ )
3	104.8	6.85 (s)	C-2,C-4	C-10,C-1'
3	104.2	6.94 (s)	C-2'',C-4''	C-10'',C-1'''
6	98.1	6.35 (d, 2.0)	C-5,C-7	C-10,C-8
8	92.8	6.76 (d, 2.0)	C-9,C-7	C-10,C-6
6''	96.4	6.73 (s)	C-5'',C-7''	C-10'',C-8''
2,6'''	128.3	8.06 (d, 8.0)	C-1',C-3',5'	C-2,C-4'
2,6'''	128.3	7.59 (d, 8.7)	C-3''',5'''	C-4'''
3,5'''	115.6	7.17 (d, 8.0)	C-4'	C-1'
3,5'''	116.0	6.74 (d, 8.7)	C-4''	C-1''
MeO-7	56.1	3.83 (s)	-	C-7
MeO-7''	56.9	3.86 (s)	-	C-7''
HO-4'''	-	10.39(s)	C-4'''	-
HO-5	-	12.83,	C-5	C-10,C-6
HO-5''	-	12.88,	C-5''	C-10'',C-6''

Chemical shift of quaternary carbons					
C	$\delta_{\text{C}}$	C	$\delta_{\text{C}}$	C	$\delta_{\text{C}}$
2	163.3	8	120.7	10	104.2
2''	163.8	7	165.3	10''	103.0
4	182.0	7'	157.3	1'	124.7
4''	182.0	9	158.4	1''	121.7
5	161.4	9''	148.3	4	161.4
5''	157.9			4''	161.3

<sup>a</sup>Homonuclear 2D- $^1\text{H}$ - $^1\text{H}$ -COSY spectra were also used in these assignments.

The cross peak of H-6''(s) with C-8'' ( $^3J_{\text{CH}}$ ) besides the chemical shift value are compatible with the quaternary oxygenated carbon in connection with the two units. The same NMR spectra analysis of acetyl

derivative (**1a**) corroborates the dimethoxylanaravone structure confirming the proposed connection C-4''-O→8'' (Table 2). Irradiation at 3.89 ( $\text{H}_3\text{CO}$ -7) yields NOE at  $\delta_{\text{H}}$  6.59(H-6) and at 6.84 (H-8); irradiation at

3.93 (H<sub>3</sub>CO-7'') afforded NOE at 6.78 (H-6'') Figure 2. The weak NOE signal observed at  $\delta_{\text{H}}$  of H-3''',5''', H-6'' and H-6 with irradiation at H<sub>3</sub>CCO (in **1a**, Figure 2) confirmed the location of three hydroxy groups at 4''', 5'' and 5 in the natural biflavone **1**. Additional NOE

was observed at the doublet of H-2',6' and of H-2''',6''' by irradiation at H-3 and H-3'', Figure 2. The cross peaks in the <sup>1</sup>Hx<sup>1</sup>H-COSY of **1a** were used to make the complete hydrogen chemical shifts assignments of the dimethyl-triacetyl-lanaraflavone derivative (Table 2).

**Table 2.** <sup>1</sup>H (400 MHz) and <sup>13</sup>C (100 MHz) NMR spectral data of **1a** in CDCl<sub>3</sub>. Chemical shifts are in  $\delta$  (ppm) and coupling constants (J) in Hz.

C	<b>1a</b>				<b>1b</b>
	HMQC ( <sup>1</sup> J <sub>CH</sub> )		HMBC		$\delta_{\text{CH}}$
	$\delta_{\text{CH}}$	$\delta_{\text{H}}$ (mult, Hz)	( <sup>2</sup> J <sub>H-C</sub> )	( <sup>3</sup> J <sub>H-C</sub> )	
3	108.3	6.52 (s)	<u>C-2</u>	C-10, C-1'	107.1
3''	107.9	6.54 (s)	<u>C-2', C-4''</u>	C-10'', C-1'''	108.1
6	107.6	6.59 (d, 2.4)	<u>C-5, C-7</u>	C-10, C-8	96.1
6''	104.8	6.78 (s)	<u>C-9', C-7''</u>	C-10, C-6	92.5
8	99.07	6.84 (d, 2.4)	<u>C-9, C-7</u>	C-10'', C-8''	92.8
2, 6'	127.3	7.82 (d, 8.8)	C-1', C-3', 5'	<u>C-2, C-4'</u>	<u>127.8</u>
2, 6''	128.0	7.45 (d, 8.8)	C-3''', 5'''	<u>C-2'', C-4''</u>	<u>127.5</u>
3, 5''	115.6	7.09 (d, 8.8)	<u>C-4'</u>	-	115.4
3, 5'''	122.3	7.05 (d, 8.8)	<u>C-4'''</u>	-	114.3
MeO-7'	56.0	3.89 (s)	-	<u>C-7</u>	<u>55.7</u>
MeO-7''	56.8	3.93 (s)	-	<u>C-7''</u>	<u>56.7</u>
H <sub>3</sub> CCO-4'''	<u>21.1/168.8</u>	2.26	H <sub>3</sub> CC=O	-	MeO-55.4
H <sub>3</sub> CCO-5	<u>21.1/169.7</u>	2.41	H <sub>3</sub> CC=O	-	MeO-56.5
H <sub>3</sub> CCO-5''	<u>21.1/169.7</u>	2.46	H <sub>3</sub> CC=O	-	MeO-55.4
Chemical shift of quaternary carbons					
	$\delta_{\text{C}}$		$\delta_{\text{C}}$		$\delta_{\text{C}}$
	<b>1a / 1b</b>		<b>1a / 1b</b>		<b>1a / 1b</b>
2	161.2 / 160.8 <sup>a</sup>		125.5 / 124.1	10	111.5 / 109.2
2''	161.5 / 160.7 <sup>a</sup>		163.5 / 164.0	10''	111.5 / 109.2
4	176.1 / 177.9		156.7 / 157.9	1	128.3 / 125.6 <sup>b</sup>
4''	176.5 / 177.5		158.8 / 159.8	1''	128.3 / 123.2 <sup>b</sup>
5	150.5 / 160.9 <sup>a</sup>		147.2 / 151.3	4	160.4 / 160.3
5''	150.5 / 156.3			4''	153.2 / 162.3

<sup>a,b</sup>can be changed.

Treatment of **1** with dimethylsulfate gave penta-O-methyl derivative (**1b**, mp 265 °C) with the same <sup>1</sup>H NMR chemical shifts in accordance with the literature.<sup>9</sup> Thus, it was possible to confirm the structure of the new lanaraflavone derivative (**1**). Also, the analysis of HMBC spectra allowed us to make the complete carbon-13 chemical shift assignment of the pentamethyl derivative **1b**, which has not been previously reported.

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