

Others Flavonoids from *Ouratea hexasperma* (Ochnaceae)

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O fracionamento cromatográfico dos extratos das folhas de *Ouratea hexasperma* (Ochnaceae) forneceu, além do novo biflavonóide 5-hidróxi-7-metoxiflavona-(4'-O-8'')-5'',4'''-diidróxi-7''-metoxiflavona, a agathisflavona, 7''-metilagathisflavona, epicatequina, 6-C-glicopiranosil-luteolina e a 3-O-glicopiranosil-quercetina já registradas na literatura. As estruturas desses compostos foram estabelecidas através da análise dos dados espectrométricos de IV, EM e RMN.

Chromatographic fractionation of the extracts from the leaves of *Ouratea hexasperma* (Ochnaceae) afforded a new flavone dimer 5-hydroxy-7-methoxyflavone-(4'-O-8'')-5'',4'''-dihydroxy-7''-methoxyflavone besides known agathisflavone, 7''-methylagathisflavone, epicatechin, 6-C-glycopyranosyl-luteolin and 3-O-glycopyranosyl-quercetin. The IR, NMR and mass spectra data analyses were used to establish the structures of these compounds.

Keywords: *Ouratea hexasperma*, Ochnaceae, flavone dimer, agathisflavone, lanaraflavone

Introduction

The genus *Ouratea* (Ochnaceae) comprises 300 tropical species occurring mainly in South America,¹ and has been reported to be used in folk medicine for the treatment of rheumatic and gastric distress. In previous reports we described the phytochemical study of species from the Ochnaceae including *Luxemburgia*²⁻⁴ and *Ouratea*⁵⁻⁸ genera. We have also isolated bisoflavonoids⁹ and a flavone dimer from *Ouratea hexasperma* collected in Amazon cerrado.¹⁰ In further investigations, we have also detected cytotoxic and antitumour activities^{11,12} and DNA topoisomerase inhibition¹² by biflavonoids from those species. Besides the biflavonoids as phytochemical constituents, it was found other pharmacological activities of *Ouratea* species.¹³⁻¹⁵ The present paper describes the isolation of a new biflavonoid, its acetyl and pentamethyl derivatives, besides the first identification of known flavonoids, agathisflavone, epicatechin, 6-C-glycopyranosyl-luteolin and 3-O-glycopyranosyl-quercetin in *O. hexasperma*. The significant amount of

7''-methylagathisflavone from the leaves of *Ouratea hexasperma* collected in Amazon cerrado¹⁰ was confirmed in this species of Mata Atlântica. The spectral data, including 1D and 2D NMR experiments of the natural substances and of the derivatives were used to establish the structures and the unambiguous ¹H and ¹³C NMR assignments.

Experimental

General procedures

Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 1605 FT-IT spectrophotometer using KBr discs or NaCl film. ¹H (200.0 MHz) and ¹³C (50.3 MHz) NMR spectra recorded on a Bruker AC 200 spectrometer using D₃CSOCD₃, D₃CCOCD₃ or CDCl₃ and TMS as internal standard. HRESIMS were obtained with a VG 7070E-HF spectrometer using (methanol:H₂O, Ar as CAD), CE 20 eV for MS and 45 eV for MS-MS) in negative mode for **1** and (methanol:H₂O) + formic acid in positive mode for **1a**. HPLC analysis was performed using a LC-6AD Shimadzu isocratic pump. Analyses were carried out in 5 mm Betasil C₁₈ preparative column 250 mm x 20 mm,

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using a mixture of methanol/water (6:4) as mobile phase, the eluted fraction at 3 mL min⁻¹ (1.0 mL injection volume) yielded a glycoside mixture. Column chromatography with silica gel (Merck and Aldrich 0.05-0.20 mm); TLC: silica gel H and G (Merck and Aldrich) were used to analyze the fractions collected from column chromatography (CC) with visualization by UV (254 and 366 nm), AlCl₃-EtOH (1%) or exposure to iodine vapor.

Plant material

The leaves of *Ouratea hexasperma* St.-Hil (Ochnaceae) were collected in João Pessoa, Paraíba State, Brazil, in October 2002. Voucher specimen (N^o JPB-21438) is deposited at the Herbarium Prof. Lauro Pires Xavier Universidade Federal da Paraíba, João Pessoa-PB, Brazil.

Extraction and isolation

Air dried leaves of *O. hexasperma* (585.0 g) were extracted exhaustively with CH₂Cl₂ and MeOH at room temperature. The solvents were removed under vacuum to yield residues **LD** (14.52 g) and **LM** (133.0 g). The **LD** residue (13.5 g) was filtered on a silica gel column using CH₂Cl₂, EtOAc and MeOH. The fraction eluted with CH₂Cl₂ (**LDD**, 7.5 g) was analyzed by TLC, IR and ¹H NMR and a mixture of aliphatic alcohols was identified. The fraction eluted with EtOAc (**LDA**, 4.0 g) was crystallized from MeOH to afford 7,7'-O-dimethylanaroflavone (**1**, mp 325-328 °C, 98.2 mg). The methanolic extract (**LM**, 133.8 g) was partitioned with hexane:diethyl ether 1:1, EtOAc and MeOH. The hexane-ether residue (**LMH**, 4.53 g) was dissolved in acetone and filtered on a sephadex LH-20 column, eluted with MeOH, to give 7'-O-methylagathisflavone (**2**, 217.0 mg), agathisflavone (**3**, 13.3 mg) and epicatechin (30.0 mg). The EtOAc residue (**LMA**, 52.2 g) was chromatographed on a silica gel column, using mixtures of chloroform and methanol, increasing the polarity to 100% methanol, to give 90 fractions. Fractions 28-31 yielded 7'-O-methylagathisflavone (**2**, 1.2 g). Fractions 53-70 (400.0 mg) were chromatographed on a silica gel column and eluted on a C₁₈ HPLC preparative column (using a Shimadzu LC-6AD isocratic pump) to yield a mixture of 6-C-glycopyranosyl-luteolin and 3-O-glycopyranosyl-quercetin (30.0 mg).

7,7''-O-dimethylanaroflavone, (**1**), C₃₂H₂₂O₁₀

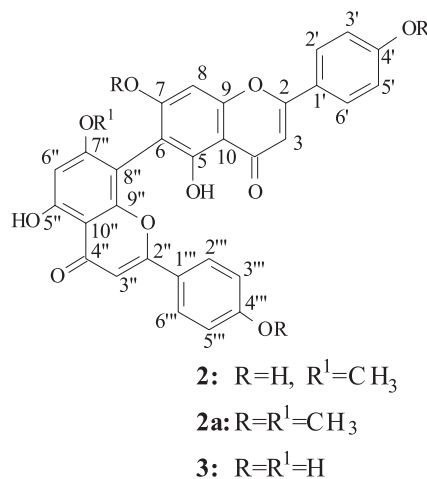
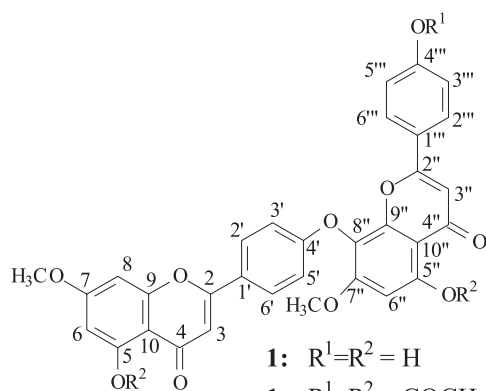
Yellow crystals, mp 325-328 °C (methanol). HRESIMS/MS: 693.1554 [M⁺⁺ + 1, 70% (C₃₈H₂₂O₁₃ + H⁺)], 443 (55), 401 (40), 361 (80) and 301 (95), (Scheme 1). ¹H and ¹³C NMR: (Table 1).

7,7''-O-dimethyl-5,5'',4''-triacetyllanaroflavone, (**1a**)

7,7''-O-dimethylanaroflavone (**1**, 33.0 mg) was dissolved in pyridine (2.0 mL) and Ac₂O (2 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). ¹H and ¹³C NMR: (Table 1).

Lanaroflavone-pentamethyl ether, (**1b**)

Compound **1** (20.0 mg), Me₂SO₄ (0.19 mL), dry Me₂CO (2.0 mL) and K₂CO₃ (0.2 g) were heated under reflux for 2 h. After removal of the inorganic salts with H₂O, the solution was dried over Na₂SO₄ and concentrated to dryness. The crude residue was subjected to silica gel column, eluting with CHCl₃ to give the penta-O-methyl derivative **1b** (mp 265 °C, 12.0 mg). ¹H NMR data:¹⁶, ¹³C NMR: (Table 1).



Results and Discussion

7''-O-methylagathisflavone (**2**), agathisflavone (**3**), epicatechin and the mixture of 6-C-glycopyranosyl-

Table 1. ¹H (200 MHz) and ¹³C (50.3 MHz) NMR data for **1** (D₃CSOCD₃), **1a** and **1b** (CDCl₃)

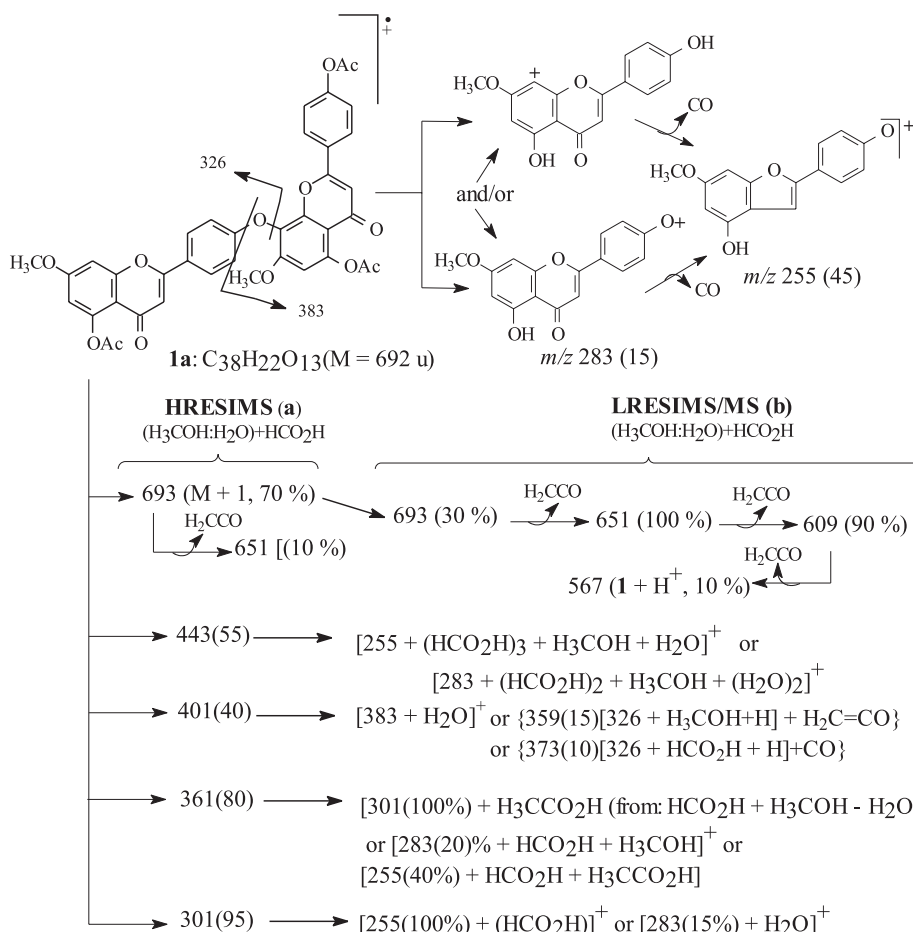
C	1		1a		1b
	δ _C	δ _H (mult, Hz) ^a	δ _C	δ _H (mult, Hz) ^a	δ _C
2	163.3	-	163.5	-	163.0
4	182.0	-	176.2	-	177.4
5	161.4	-	158.8	-	166.2
7	165.3	-	163.5	-	164.0
9	158.4	-	160.4	-	157.9
10	104.2	-	109.1	-	108.1
1'	124.7	-	128.4	-	125.5
4'	161.4	-	161.2	-	160.9
2''	163.8	-	161.5	-	160.7
4''	182.0	-	176.5	-	177.4
5''	157.9	-	153.2	-	162.2
7''	157.3	-	150.5	-	156.3
8''	120.7	-	125.5	-	123.2
9''	148.3	-	147.2	-	146.8
10''	103.0	-	107.9	-	107.3
1'''	121.7	-	125.8	-	124.0
4'''	161.3	-	156.1	-	166.1
H ₃ CCO-4''''	-	-	168.9	-	-
H ₃ CCO-5,5''	-	-	169.70x2	-	-
CH					
3	104.8	6.85 (s)	108.3	6.52 (s)	108.1
6	98.1	6.35 (d, 2.0)	107.6	6.59(d, 2.4)	96.1
8	92.8	6.76 (d, 2.0)	99.07	6.84 (d, 2.4)	92.8
2',6'	128.3	8.06 (d, 8.0)	127.3	7.82 (d, 8.8)	127.5
3',5'	115.6	7.17 (d, 8.0)	115.6	7.09 (d, 8.8)	114.4
3''	104.2	6.94 (s)	107.9	6.54 (s)	107.1
6''	96.4	6.73 (s)	104.8	6.78 (s)	92.4
2''',6'''	128.3	7.59 (d, 8.7)	128.0	7.45 (d, 8.8)	127.8
3''',5'''	116.0	6.74 (d, 8.7)	122.3	7.05 (d, 8.8)	115.4
CH ₃					
MeO-7	56.1*	3.83 (s)	56.0*	3.89 (s)	56.5*
MeO-7''	56.9*	3.86 (s)	56.8*	3.93 (s)	56.6*
MeO-5	-	-	-	-	55.8*
MeO-5''	-	-	-	-	56.7*
MeO-4''	-	-	-	-	55.4*
H ₃ CCO ₂ -4''', 5H ₃ CCO ₂ -5''	--	--	21.1, 21.121.1	2.26(s), 2.41(s)2.46(s)	-
HO-5,5'', 4''''	-	12.83, 12.88, 10.39(s)	-	-	-

^aHomonuclear 2D-¹H-¹H-COSY spectra were also used in these assignments; *Values can be interchanged.

luteolin and 3-O-glycopyranosyl-quercetin were identified by ¹H and ¹³C NMR spectral analysis of the natural substances and comparison of the data with values described in the literature.^{10,17-19} The ¹H - {¹H}-NOE spectra resulting from irradiation of H-3, 3'', 6'' (in **2**, **2a** and **3**) showed nOe of H-2',6', 2''',6''', HO-5'' and H₃CO-7'', in the respective structures; from irradiation of the methoxy groups (in **2**, **2a**) were detected NOE in the signals of H-6'' (**2** and **2a**), H-3',5' and H-3''',5''' (**2a**). This information and the absence of NOE in the signals of H₃CO- and of HO-5 in the spectra from irradiation of H-8 (in **2**) confirmed the structures of these biflavonoids.

The IR spectrum of **1** exhibited absorption bands at max 3420 cm⁻¹ (OH), 1658 cm⁻¹ (conjugated carbonyl) and 1600, 1500 and 1440 cm⁻¹ (aromatic ring). The peak at *m/z* 566 (2%, M⁺) observed in the LREIMS and the ¹H and

¹³C (BBD and DEPT) NMR spectral analysis was used to make the expanded molecular formula (C=O)₂ C₁₅(CH)₁₃(OMe)₂(OH)₃O₃ = C₃₂H₂₂O₁₀. This formula is consistent with a biflavonyl ether skeleton with one free hydroxyl group (δ_H 10.39), two H-bonded hydroxy groups (δ_H 12.83 and 12.88), two methoxy groups (δ_H 3.83 and 3.86, s, 3H), four doublets at δ_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 δ_H 6.94, 6.85 and 6.73 and two doublets (2.0 Hz) at 6.35 and 6.76. The δ_{CH} at 104.8, 104.2, 98.1, 96.4 and 92.8 are compatible with C-3, -3', -6, -8 and -6'' of **1**. Comparison of the carbon chemical shifts of **1** with those of 7-methylflavanone registered in the literature⁸ revealed the shielding of the methine carbons CH-6 (98.1), CH-8 (92.8), CH-6'' (96.4) through a γ effect of the carbon atom in the O-methyl group



Scheme 1.

Table 2. ¹H{¹H}-nOe difference spectral data of **1** (DMSO-d₆) and **1a** (CDCl₃)

¹ H	Irradiation		nOe	
	δ_H		H; δ_H (%)	
	1	1a	1	1a
AcO-4'''	-	2.26	-	3''', 5'''; 7.05(0.5)
AcO-5	-	2.41	-	6; 6.59(0.5)
AcO-5''	-	2.46	-	6''; 6.78(0.2)
MeO-7	3.83	3.89	6; 6.35(4.0)8; 6.76 (2.0)	6; 6.59(11.0)8; 6.84(13.0)
MeO-7''	3.86	3.93	6''; 6.73(5.0)	6''; 6.78(10.0)
3	-	6.52	-	2', 6'; 7.82(20.0)
3''	-	6.54	-	2''', 6'''; 7.45(25.0)

at C-7 and C-7''. The absence of additional signal of CH near 93.0 ppm indicated that C-8 is involved in the connection of the flavonoid moieties. The ¹H{¹H}-nOe difference spectra of **1** with irradiation at 3.86 showed nOe at δ_H 6.73(s) and irradiation at 3.83 showed NOE at 6.76(d, 2 Hz) and 6.35 (d, 2.0 Hz) (Table 2). The LRESIMS-MS (negative mode) of ion with *m/z* 565 (40%) yields peaks at

550(60%), 283(100%) and 255(50%) that are in agreement with dimethoxyflavone. These 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'' and also the 4'-O-8 connection. The homonuclear 2D ¹Hx¹H-COSY was used to make the chemical shift assignment of **1** (Table 1). The analysis of 2D ¹Hx¹H-COSY

and $^1\text{H}\{^1\text{H}\}$ -nOe difference spectra (Table 2) together with the peaks at m/z (%) 693.1554 [$\text{M}^+ + 1$, 70% ($\text{C}_{38}\text{H}_{22}\text{O}_{13} + \text{H}^+$)], 443 (55), 401 (40), 361 (80) and 301 (95) in the HRESIMS (positive mode, Scheme-1) of the acetyl derivative (**1a**) corroborates the dimethoxylanarafflavone structure. Irradiation at 3.89 (H_3CO -7) yields an NOE at δ_{H} 6.59 (H-6) and 6.84 (H-8) and irradiation at 3.93 (H_3CO -7") afforded NOE at 6.78 (H-6"). The nOe observed at the chemical shifts of H-3""",5""", H-6"" and H-6 with irradiation at H_3CCO (in **1a**) confirmed the location of three hydroxy groups at 4""", 5"" and 5 in the natural biflavone **1**. Additional NOE observed at the doublet of H-2',6' and of H-2""",6"" by irradiation at H-3 and H-3"" (Table 2) and the cross peaks in the $^1\text{Hx}^1\text{H}$ -COSY of **1a** were used to make the complete hydrogen chemical shifts assignments (Table 1). The LRESIMS-MS (positive mode) spectra of the ion with m/z 693 allowed us to confirm the three acetyl group with peaks at m/z (%): 651 (100), 609 (90) and 567 (10). The same analysis was made with ions (set of species: fragment of **1a**, formic acid, methanol and water) at m/z 443, 401, 361 and 301 to identify the peaks of **1a** with m/z 383, 326, 283 and 255 (Scheme 1). Treatment of **1** with dimethylsulfate gave the penta-O-methyl derivative (**1b**, mp 265 °C) with the same ^1H NMR chemical shifts registered in the literature¹⁶. Thus, it was possible to confirm the structure of the new lanarafflavone derivative (**1**) and to make the complete carbon-13 chemical shift assignment of the pentamethyl derivative **1b**, which has not been previously reported (Table 1).

Acknowledgements

The authors are grateful to CNPq, CAPES and FAPERJ for scholarships and financial support.

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Received: March 2, 2004

Published on the web: March 4, 2005

FAPESP helped in meeting the publication costs of this article.