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Artigo

Biflavones and triterpenoids isolated from *Ouratea castaneifolia* (DC.) Engl., Ochnaceae

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RESUMO: "Biflavonas e triterpenóides isolados de *Ouratea castaneifolia* (DC.) Engl., Ochnaceae". O presente trabalho trata da investigação química das folhas e caule da espécie *Ouratea castaneifolia* (DC.) Engl., sobre a qual não há registros de estudos químicos ou farmacológicos anteriores. O estudo fitoquímico clássico dos extratos orgânicos do caule e das folhas de *O. castaneifolia* foi aliado à técnica da cromatografia líquida de alta eficiência (CLAE) e resultou na identificação de dezessete metabólitos: sete triperpenos (friedelina, 3β-friedelinol, α-amirina, β-amirina, lupeol, taraxerol e germanicol), quatro esteróides (sitosterol, estigmasterol e os glucosídeos sitosteril 3-*O*-β-D-glicopiranosídeo e estigmasteril 3-*O*-β-D-glicopiranosídeo), uma isoflavona (5,7,4´-trimetoxiisoflavona), uma flavona (5,4´-diidroxi-7,3´,5´-trimetoxiflavona), quatro biflavonas (amentoflavona, 7,7"-O-dimetil-amentoflavona, heveaflavona e tetrametilamentoflavona). A identificação das substâncias foi feita com base na análise de espectros de RMN de ^{1}H, ^{13}C e técnicas bidimensionais. As classes dos metabólitos identificados estão de acordo com aquelas citadas em estudos químicos do gênero *Ouratea.*

Unitermos: *Ouratea castaneifolia,* Ochnaceae, biflavonas, flavonóide, terpenóide.

ABSTRACT: This paper presents the chemical investigation of the leaves and stems of *Ouratea castaneifolia* (DC.) Engl.. There are no chemical or pharmacological studies with this species. Classic phytochemical investigation of the organic extracts together with high pressure liquid chromatography (HPLC) procedures lead to the identification of seventeen metabolites: seven triterpenes (friedelin, 3β-friedelinol, α-amyrin, β-amyrin, lupeol, germanicol and taraxerol), four steroids (sitosterol, stigmasterol and the glycosides sitosteryl 3-*O*-β-D-glucopyranoside and stigmasteryl 3-*O*-β-D-glucopyranoside), one isoflavone (5,7,4'-trimethoxyisoflavone), one flavone (5,4'-dihydroxy-7,3',5'-trimethoxyflavone) and four biflavones (amenthoflavone, 7,7"-O-dimethylamenthoflavone, heveaflavone and tetramethylamenthoflavone). The structures of the compounds were established by the analysis of ^{1}H, ^{13}C NMR spectra including bidimensional techniques. The classes of the identified metabolites are in agreement with previous studies of the *Ouratea* genus.

Keywords: *Ouratea castaneifolia*, Ochnaceae, biflavones, flavonoid, terpenoid.

INTRODUCTION

Ouratea and other genera of Ochnaceae are a rich source of flavonoids and biflavonoids and according to Suzart et al. (2007), the biflavonoids can be used as chemotaxonomic markers of the genus *Ouratea* as well as of *Luxemburgia* from the same botanic family. Lignans, triterpenes, diterpenes, steroids, monosaccharide, depsides, triglycerides and chloroisoflavones were also reported from this genus (Velandia et al., 1998a and 1998b; Carvalho et al., 2000; Manga et al., 2001; Mbing et al., 2003a and 2003b; Felício et al., 2004; Estevam et al., 2005). Some biflavonoids, as well as extracts of the *Ouratea* species showed important biological activities, as was previously published (Suzart et al., 2007).

Ouratea castaneifolia (DC.) Engl. Ochnaceae

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was collected in Marajó Island in the North of Brazil. This species is known in the Amazon region of Brazil as "farinha-seca", "mangue-do-mato" or "pau-de-serra" and its wood is used in small constructions and its bark is tonic and adstringent and contains tannins (Le Cointe, 1934). This work describes the phytochemical study of the extracts from the leaves and stems of *O. castaneifoila.*

MATERIAL AND METHODS

General procedures

Melting points are uncorrected. NMR spectra were recorded on a Mercury-300 Varian spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) using the solvent (DMSO-d_6, Me_2CO-d_6, CD_3OD or $CDCl_3$) and TMS as internal standard. Silica gel (Merck and Vetec 0.05-0.20 mm) or Sephadex LH-20 was used on separations by column chromatography (CC). Silica gel HF (Merck) was used for TLC and revealed by UV (254 and 366 nm), acid solution of ceric sulphate and exposure to iodine vapor. HPLC-UV analyses were performed using a Shimadzu (Shimadzu, Tokyo, Japan) liquid chromatography modular system consisting of two LC-10AD pumps, an UV-Vis Shimadzu SPD-10AV detector, and an LC Workstation Class LC-10 system for data processing. The samples were introduced using an injection valve fitted with 20 µL loop (Rheodyne, California, USA). The mobile phase consisted of water:methanol:acetonitrile (24:40:36, isocratic mode) at a flow rate of 1 $mL.min^{-1}$. A C18 column (Gemini, 250 mm x 4.6 mm x 5 µm) fitted with guard column (Gemini, C18, 4 mm x 3 mm x 5 µm) was utilized. UV detection was performed at 330 nm.

Plant material

Leaves and stems of *Ouratea castaneifolia* (DC.) Engl. Ochnaceae were collected in Salvaterra (Marajó Island, State of Pará, Brazil) and identified by one of the authors (S.T.R.). A voucher specimen (IAN 172171) is deposited at the herbarium of Embrapa-Amazônia Oriental (Belém, Brazil).

Extraction and isolation

Dried and powdered leaves (2500 g) and stems (1880 g) were extracted with *n*-hexane, dichloromethane and methanol by percolation at room temperature. The solutions of leaves (L) and stems (S) were concentrated under vacuum to yield the *n*-hexane (H), dichloromethane (D) and methanolic (M) extracts from the leaves (extract LH: 28.25 g, extract LD: 51.85 g and extract LM: 238.10 g) and stems (extract SH: 3.40 g, extract SD: 2.30 g, extract SM: 32.00 g). Extracts LH (28.25 g), LD (25.00 g) and SD (2.30 g) were fractionated by CC on silica gel using mixtures of hexane with EtOAc and MeOH gradually increasing polarity as elluents. The fractions were purified using similar CC procedures.

The fractions from LH extract eluted with *n*-hexane:EtOAc (6:8) afforded two mixtures of substances, one of compounds **7-11** (102 mg) and the other of **12-13** (11 mg). The fraction from LD extract eluted with EtOAc (100%), named as BF, was analyzed by ^{1}H NMR spectrum and characteristics signals of a mixture of biflavonoids were identified. After successive CC procedures, including the use of Sephadex LH-20 and methanol as elluent, no separation was achieved; fraction BF was than submitted to HPLC (chromatogram on Figure 1) yielding compounds **14** (4 mg), **15** (4 mg), **16** (4 mg) and **17** (7 mg). Compound **4** was further identified by HPLC from fraction BF. Part of the extract LM (20 g) was suspended on MeOH:H_2O (3:1) and successively extracted with $CHCl_3$, EtOAc and *n*-BuOH yielding the $CHCl_3$, EtOAc and *n*-BuOH phases. During concentration of the $CHCl_3$ phase from LM extract, a pale yellow solid precipitated and was washed with methanol affording compound **17** (53 mg). The HPLC chromatogram of the CHC_{l3} phase from methanolic extract partition was similar to BF fraction.

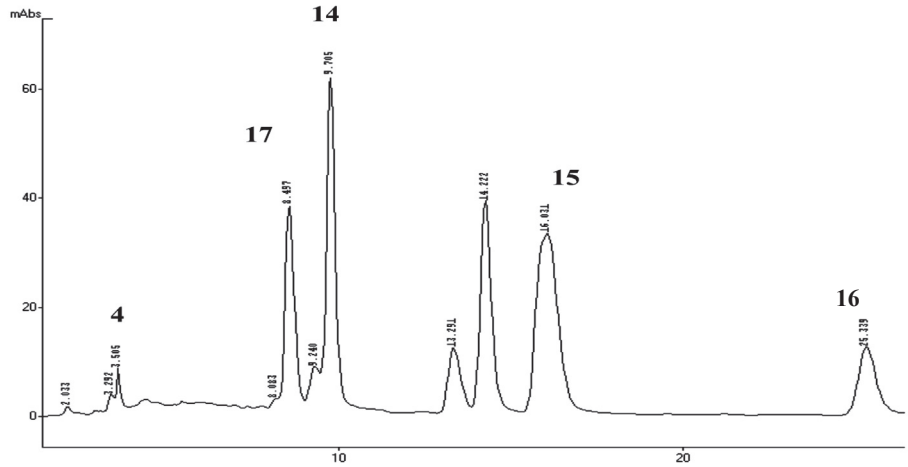


Figure 1. Chromatogram of fraction BF from *Ouratea castaneifolia* (DC.) Engl., Ochnaceae, eluted with H_2O:MeOH:MeCN (24:40:36). Flow 1 mL/min. UV Detection: λ=330 nm.

During the concentration of the *n*-hexane solution of the stems (solution SH), a solid material precipitated and was purified by recristalization with *n*-hexane and EtOAc affording a mixture of substances **1** and **2** (16 mg). Repeated CC procedures of the fractions of extract SD elluted with *n*-hexane:EtOAc (2:8) afforded compound **3** (25 mg); the fraction elluted with *n*-hexane:EtOAc(1:1) yielded compound **4** (5 mg). The fractions from the SM extract (32.00 g) were purified by CC eluting with hexane:EtOAc (25:75) and EtOAc:MeOH (9:1) affording additional quantities of compound **10** (8 mg) and a mixture of compounds **5** and **6** (20 mg).

RESULTS AND DISCUSSION

The chemical study of the stems of *Ouratea castaneifolia* (DC.) Engl. Ochnaceae lead to the identification of the triterpenes friedelin (**1**) (Mahato & Kundu, 1994) and 3β-friedelinol (**2**) (Salazar et al., 2000), the isoflavone 4',5,7-trimethoxyisoflavone (**3**) (Jha et al., 1980; Wang, 2005) and the flavone 4',5-dihydroxy-3',5',7-trimethoxyflavone (**4**) (Zahir et al., 1996) and two glycosides **5** and **6** identified sitosteryl and stigmasteryl 3-*O*-β-D-glucopyranosides (Chaurasia & Wichth, 1987), respectively.

Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of **1** in DMSO-d_6. Chemical shifts are in δ (ppm) and coupling constants (J) in Hz.

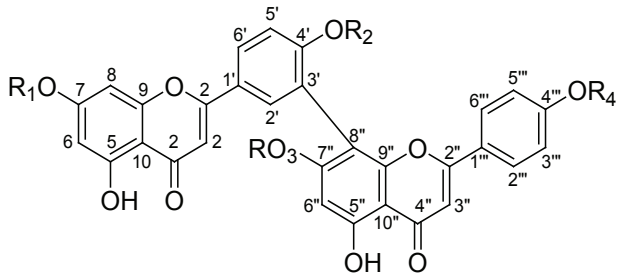
C	HMQC ($^{1}J_{CH}$)		*HMBC* ($^{2,3}J_{H-C}$)
	δ_{CH}	δ_H (mult,Hz)	
3	103.3	6.76(s)	C-2,C-4,C-10,C-1'
3"	102.7	6.68(s)	C-2",C-4",C-10",C-1"'
6	98.2	6.31(d, 2.4)	C-7,C-8,C-10
8	92.9	6.66(4, 2.4)	C-6,C-9,C-10
6"	95.6	6.60(s)	C-7",C-8",C-10"
2'	131.5	8.09(s, 2.4)	C-2,C-4',C-6',C-8"
5'	128.3	7.26(d, 8.7)	C-1',C-3'
6'	128.2	8.05(dd, 8.7, 2.4)	C-2'
2"',6"'	128.4	7.65(d, 8.7)	C-3"',5"',C-2",C-4"'
3"',5"'	116.0	6.84(d, 8.7)	C-2"',6"',C-1"'
MeO-7	56.6	3.82(s)	C-7
MeO-7"	56.2	3.83)s)	C-7"
HO-5	-	12.95(s)	C-6,C-10
HO-5"	-	13.24(s)	C-6",C-10"

Chemical shift of quaternary carbons

C	δ_C	C	δ_C	C	
2	164.2	8"	105.0	10"	104.2
2"	16164.2	7	165.3	1'	121.1
4	182.1	7"	162.8	3'	119.8
4"	182.5	9	157.7	4'	159.6
5	161.4	9"	153.7	1" '	121.3
5"	161.5	10	104.8	4"'	161.3

[a]Homonuclear 2D-^{1}H-^{1}H-COSY spectra were also used in these assignments.

The triterpenes, α-amyrin (**7**), β-amyrin (**8**), lupeol (**9**), taraxerol (**10**) and germanicol (**11**) (Mahato & Kundu, 1994) and the steroids mixture, sitosterol (**12**) (Nes et al., 1992) and stigmasterol (**13**) (Forgo & Kövér, 2004), were identified in the extract from the leaves of *O. castaneifolia*. The biflavones **14-17** were also isolated from the leaves of the plant and were identified as 7,7"-di-*O*-methylamenthoflavone (Gu et al., 1990), heveaflavone (4"',7,7"-tri-*O*-methylamenthoflavone) (Carbonezi et al., 2007), 4',4"',7,7"-tetra-*O*-methylamenthoflavone (Markham et al., 1987) and amenthoflavone (Dora & Edwards, 1991; Markham et al., 1987), respectively.



	R_1	R_2	R_3	R_4
14	Me	H	Me	H
15	Me	H	H	H
16	Me	Me	Me	Me
17	H	H	H	H

Compound **3** was isolated from *O. hexasperma* (Moreira et al., 1994) and heveaflavone (**15**) from *O. multiflora* (Carbonezi et al., 2007); amenthoflavone (**15**) is common in *Ouratea* (Felicio et al., 2004; Felicio et al., 2001; Velandia et al., 2002). To our knowlegment, this is the first occurrence of germanicol (**11**), 4',5-dihydroxy-3',5',7-trimethoxyflavone (**4**) and of the biflavone 7,7"-di-*O*-methylamentoflavone (**14**) in the *Ouratea* genus.

All the NMR data of compound **15** (heveaflavone) were in agreement with the literature data (Carbonezi et al., 2007), except for the attribution of the HO-5 and HO-5" that were changed. In the HMBC of **15**, the signal at δ_H 12.95 (OH-5) showed ^{2,3}JCH correlation with δ_C 98.2 (C-6) and the signal at δ_H 13.21 (OH-5") showed ^{2,3}JCH correlation with δ_C 95.6 (C-6").

The ^{13}C NMR data of compound **14** are being published for the first time. It were made by the 1D (BBD and DEPT) and 2D (HMQC and HMBC) NMR experiments analysis (Table 1). The ^{1}H NMR spectra [1D and 2D (^{1}H-^{1}H-COSY)] of compound **14** showed signals of two chelated hydroxyls (δ_H 12.95 and 13.24), hydrogens of two aromatic methoxyl groups, two hydrogens of ring A (*meta* coupled), one non coupled hydrogen (ring A"), the hydrogens of a 1,3,4-trisubstituted aromatic ring (ring B) and the hydrogens of a *para*-substituted aromatic ring (Ring B'"). These ^{1}H NMR data are in accordance to those published by Gu et al. (1990). The ^{13}C NMR spectrum of compound **14** showed thirty signals, ten signals were attributed to twelve sp^2 CH (including the two signals atributted to C-2'" and C-6'" and to C-3'" and C-5'"), two sp^3 carbons (δ_{CH3} 56.2 and 56.6), sixteen sp^2 quaternary carbons and two carbonyl groups (δ_C 182.1 and 182.5). The structure of **14** was confirmed on the basis of HMBC spectrum (Table 1, Figure 1), which showed heteronuclear long range couplings ^{2,3}JCH of H-2' with C-8" and of H-6" with C-8". These data together with the absence of

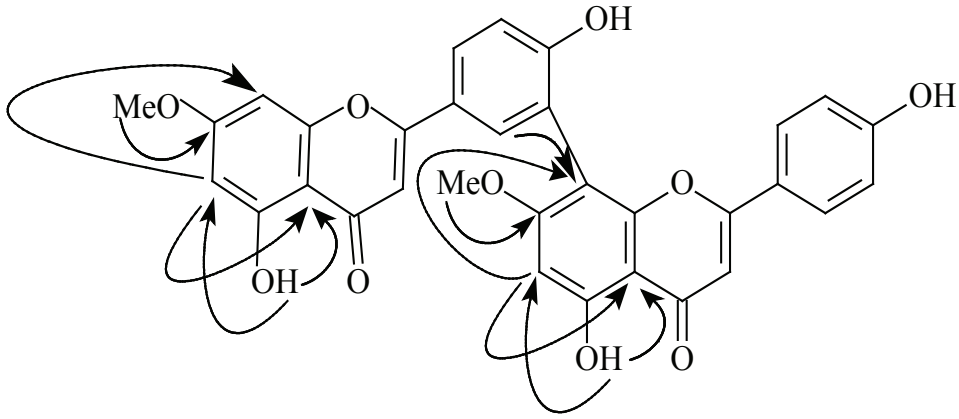


Figure 2. Some $^{2,3}J_{CH}$ long-range correlations on the HMBC of compound **14**.

correlations with C-9", are characteristic of biflavonoids with an amenthoflavone skeleton (Goh et al., 1992). The localization of the methyl groups on compound **14** was deduced by comparison of the chemical shifts on the ^{13}C-NMR spectra with those of amenthoflavone taking in account the protection effect caused by the presence of these groups on the vicinal carbons as described in the literature (Markham et al., 1987). The HMBC experiments also showed correlations of the quaternary carbons C-7" (δ_C 162.8) and C-7 (δ_C 165.2) with the hydrogens of the methoxyl groups at δ_H 3.83 (OMe-7") and 3.82 (OMe-7), respectively.

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