Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
19(1A): 33-35, Jan./Mar. 2009

Received 17 July 2008; Accepted 9 December 2008

A new derivative of dihydroochnaflavone isolated from *Luxemburgia* species (Ochnaceae) and the complete ^{1}H and ^{13}C NMR chemical shifts assignments

Mario G. de Carvalho*, Virginia C. Silva, José G. da Rocha Jr

Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, BR 465 KM 07, 23890-000, Seropédica-R.J, Brasil

RESUMO: "Um novo derivado da dihidroochnaflavona isolada de *Luxemburgia* species (Ochnaceae) e o completo assinalamento dos dados de RMN de ^{1}H e ^{13}C". O biflavonóide 2",3"-diidroochnaflavona, isolada de espécies de *Luxemburgia* (Ochanaceae), foi tratada com diazometano e forneceu o éter trimetílico que, em seguida, foi tratado com anidrido acético/piridina obtendo-se um novo derivado, 5-acetil-7,4'-dimetil-flavona-(3'→*O*-4'")-5"-acetil-7"-metil-flavanona. Fez-se o completo assinalamento dos dados de RMN ^{1}H e ^{13}C através da análise dos espectros uni- e bidimensionais do derivado.

Unitermos: Ochnaceae, *Luxemburgia*, biflavona, trimetil-diacetil-diidroochnaflavona.

ABSTRACT: The biflavonoid 2",3"-dihydroochnaflavone, isolated from the *Luxemburgia* species (Ochanaceae), was treated with diazomethane to obtain the trimethyl-ether that was treated with pyridine/acetic anhydride to yield a new derivative, 5-acetil-7,4'-dimethyl-flavone-(3'→*O*-4'")-5"-acetil-7"-methyl-flavanone. The complete ^{1}H and ^{13}C NMR data assignments of the new derivative were made by the one- and two-dimensional spectral analysis.

Keywords: Ochnaceae, *Luxemburgia*, biflavone, trimethyl-diacetyl-dihydroochnaflavone.

INTRODUCTION

The antitumor and cytotoxic activities of biflavonoids isolated from *Luxemburgia* species have been evaluated. Two natural biflavonoids 2",3"-dihydroochnaflavone and luxenchalcone was cytotoxic to murine Ehrlich carcinoma and human cells lines. The acetyl and the methyl derivatives were not cytotoxic to the analyzed tumour cells lines (Daniel et al., 2007; Oliveira et al., 2005). Species of *Ochna, Ouratea* and *Luxemburgia* (Ochnaceae) are great source of these natural biflavonoids (Likhitwitayawuid et al., 2001; Carvalho et al., 2002, 2004; Carbonezi et al., 2007). Besides the structural determination, we have analyzed the spectral data of the natural biflavonoids and their new derivatives (Carvalho et al., 2006a,b; Carvalho et al., 2003, Carvalho and Daniel, 2005). In this paper we describe the preparation and the complete proton and carbon-13 NMR assignments of a new derivative of 2",3"-dihydroochnaflavone.

MATERIAL AND METHODS

General procedures

Melting points are uncorrected. Fourier transform NMR spectra were recorded on a Varian INOVA-500 model spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The NOE-difference experiment was recorded on a Bruker AC200 spectrometer operating at 200 MHz for ^{1}H. Samples were analyzed in 5 mm tubes, using $CDCl_3$ as solvent containing tetramethylsilane as internal standard. The natural biflavone (**1**) was isolated from the leaves of *Luxemburgia nobilis* and from *L. octandra* as described in the literature (Carvalho et al., 2002; Carvalho et al., 2004).

Preparation of 5,5"-diacetyl- 7,4',7"-trimethyl-2",3"-dihydroochnaflavone (1b)

The new derivative **1b** was prepared by treating a methanol solution of the natural biflavone **1** (50.0 mg) with ethereal diazomethane solution prepared as the procedure described by Carvalho et al (2006b). After evaporation of the solvents, the residue was dissolved in acetone and purified by CC on silica gel. The fraction eluted with acetone yielded **1a** (gum, 48.0 mg) identified as a trimethyl derivative. The product **1a** was dissolved in 3.0 mL of Ac_2O:pyridine (1:1) solution and refluxed at 60 °C overnight. Usual work-up yielded **1b** (gum, 32.0 mg), Figure 1.

---

* *E-mail: mgeraldo@ufrrj.br, Tel./Fax +55-21-2682-2807*

RESULTS AND DISCUSSION

The number of methoxyl groups detected by ^{1}H and ^{13}C NMR spectra of the derivative **1a** let us to confirm the dimmers structure. If it was a mixture of monomers (flavone and flavanone) this product would have five methoxyl groups. The analysis of ^{13}C NMR (BBD and DEPT) spectra of compound **1b** led us to identify twelve sp^{2} CH including two signals at δ_{CH} 127.9 and 116.3, each representing two carbon atoms; signals of sp^{3} carbons at δ_{CH} 79.1 and δ_{CH2} 44.9 besides methoxyl and methyl groups; fourteen quaternary sp^{2} carbons (4xC and 10xC-O) and four carbonyl groups at δ_{C} 188.7, 176.5, 169.5 and 169.4 were identified. In the ^{1}H NMR spectrum were identified ten protons of aromatic ring including two sets of *meta*-coupled doublets (^{1}Hx^{1}H-TOCSY) at δ_{H} 6.29 and 6.44 (*J* = 2.5 Hz) and at δ_{H} 6.65 and 6.87 (*J* = 2.0 Hz) which belong to the 6, 8, 6" and 8" protons of two flavonoid moieties (Table 1). The paramagnetic larger shift of the protons H-8/H-8" in comparison with H-6/6", reveals that, when the acetyl group is sterically hindered, the effect in *para* is more pronounced than in *ortho* effect in consequence of the shielding effect of the carbonyl group, the widespread statement when it is attributed major *ortho* effect of acetyl (Carvalho & Braz-Filho, 1993). The presence of a singlet at δ_{H} 6.76 (H-3) and the double doublets at δ_{H} 5.45 (J_{1} = 13.5 and J_{2} = 3.0 Hz; H-2"), 3.04 (J_{1} = 16.5 and J_{2}= 13.5 Hz; H-3"ax) and at δ_{H} 2.75 (J_{1} = 16.5 and J_{2} = 3.0 Hz; H-3"eq) were similar to those of **1** and **1a**. The chemical shifts of ring B proton in the flavone moiety was identified by the signals at δ_{H} 7.13 (*d*, *J* = 8.7 Hz, H-5'), 7.58 (*d*, *J* = 2.0 Hz, H-2') and 7.75 (*dd*, J_{1} = 7.8 and J_{2} = 2,0 Hz, H-6') Table 1. The AA'BB' system was presented by two doublets (*J*=

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **1b.**

C	HMQC ($^{1}J_{HC}$)		HMBC ($^{2,3}J_{HC}$)
	δ_{C}	δ_{H}	
2	162.2	-	-
3	106.8	6.76 (*s*)	C-10, C-4, C-2, C-1'
4	176.5	-	-
5	151,.9	-	-
6	104.8	6.29 (*d*; 2.5)	C-5, C-7, C-10
7	165.6	-	-
8	99.5	6.44 (*d*; 2.5)	C-9, C-7
9	163.0	-	-
10	108.0	-	-
1'	123.9	-	-
2'	119.6	7.58 (*d*; 2.0)	C-1', C-2', C-4'
3'	144.7	-	-
4'	154.7	-	-
5'	112.9	7.13 (*d*; 7.8)	C-4', C-3', C-6'
6'	124.1	7.75 (*dd*; 2.0 and 7.8)	C-2, C-4', C-2'
2"	79.1	5.45 (*dd*; 3.0 and 13.5)	C-3"
3"	44.9	2.75 (*dd*; 3.0 and 16.5) 3.04 (*dd*; 13.5 and 16.5)	C-4", C-2", C-1"'
4"	188.7	-	-
5"	150.4	-	-
6"	108.8	6.65 (*d*; 2.0)	C-5", C-7"
7"	164.2	-	-
8"	98.9	6.87 (*d*; 2.0)	C-9", C-7"
9"	158.8	-	-
10"	113.0	-	-
1"'	132.8	-	-
2"',6"'	127.9	7.42 (*d*; 8.5)	C-4"', C-3"',5"'
3"',5"'	116.3	7.0 (*d*; 8.5)	C-4"', C-1"', C-2"',6"'
4"'	158.1	-	-
7-OCH$_{3}$	55.7	3.83 (*s*)	C-7
7"-OCH$_{3}$	56.2	3.92 (*s*)	C-7"
4'-OCH$_{3}$	56.2	3.92 (*s*)	C-4'
C=O (5)	169.4	-	-
C=O (5")	169.5	-	-
CH$_{3}$ (5)	21.1	2.43 (*s*)	C=O
CH$_{3}$ (5")	21.1	2.39 (*s*)	C=O

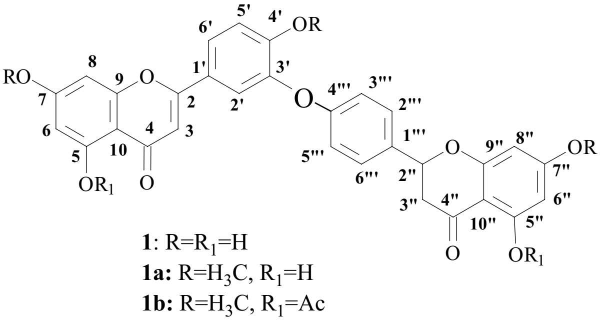

Figure 1. Structures of 2”,3”-dihydro-ochnaflavone and its derivatives.

8.5 Hz, 2H each) at δ 7.42 and 7.0 which were assigned to H-2’’’,6’’’ and H-3’’’,5’’’ of the flavanone moiety, respectively. The cross peaks of heteronuclear long-range couplings observed in the ^{13}Cx^{1}H-COSY-$^nJ_{CH}$ (n = 2 and 3, HMBC) spectra of **1b** were used to identify the chemical shift of quaternary carbons (Table 1). The signals of H-3 with C-10, C-4, C-2, C-1’; of H-6 with C-5, C-7 and C-10 besides signals of H-8 with C-7, C-9 and C-10 confirmed the chemical shifts of quaternary carbons of ring A of the flavone moiety (Table 1). The spectra obtained from NOEDIFF-NMR experiments of **1b** showed NOE signals at δ 7.13 (*d*, H-5’), 6.29 (*d*, H-6), 6.44 (*d*, H-8), 6.65 (*d*, 2.0 Hz, H-6”) and 6.87 (*d*, H-8”) with irradiation at the methoxy groups; additional NOE signals at δ at 3.04 (H-3a), 2.75 (H-3b) and at 7.42 (H-2’’’,6’’’) with irradiation in the frequency of H-2” (δ_H 5.45) confirmed the AA’BB’ system located in the flavanone moiety. These observations confirmed the C-3’→O-C-4’’’ connection between the flavone and flavanone moieties. The comparison of the ^{13}C NMR spectral data with those of **1** (Carvalho et al., 2002), along with the analysis of the ^{13}Cx^{1}H-COSY, $^nJ_{CH}$ (n = 1, HMQC, n = 2 and 3, HMBC), allowed to define the structure and make the complete proton and carbon-13 NMR chemical shift assignments of the new biflavonoid derivative 5,5”-diacetyl-(3’-O-4’’’)-7,4’,7”-trimethyl dihydroochnaflavone (**1b**, Table 1).

ACKNOWLEDGEMENTS

The authors are grateful to CNPq, FAPERJ and CAPES for a research fellowship and for financial supports and to Instituto de Química/UNESP/Araraquara for the 500 MHz NMR spectra.

REFERENCES

Carbonezi CA, Hamerski L, Gunatilaka AAL, Cavalheiro A, Castro-Gamboa I, Silva DHS, Furlan M, Young MCM, Lopes MN, Bolzani VS 2007. Bioactive flavone dimers from *Ouratea multiflora* (Ochnaceae). *Rev Bras Farmacogn 17*: 319-324.

Carvalho MG, Braz-Filho R 1993. Novas técnicas de RMN e os deslocamentos químicos dos átomos de ^{1}H e ^{13}C da isoflavana duartina. *Quim Nova 16*: 89-94.

Carvalho MG, Oliveira MCC, Silva CJ, Werle AA 2002. New biflavonoid and other constituents isolated from *Luxemburgia nobilis*. *J Braz Chem Soc 13*: 119-123.

Carvalho MG, Alves CCF, Santos VMR, Schripsema J 2003. O completo assinalamento de RMN ^{1}H e ^{13}C de novos derivados da luxenchalcona isolada de *Luxemburgia octandra*. *Rev Univ Rural, Ser Ci Exatas e da Terra 22*: 89-95.

Carvalho MG, Alves CCF, Silva KGS, Werle AA, Eberlin MN 2004. Luxenchalcone, a new bichalcone and other constituents from *Luxemburgia octandra*. *J Braz Chem Soc 15*: 146-149.

Carvalho MG, Daniel JFS 2005. ^{1}H and ^{13}C-NMR assignments of two new derivatives from lanaraflavone, a 4’-O-8” biflavone dimer from *Ouratea hexasperma* (Ochnaceae) *Ann Magn Reson 4*: 29-34.

Carvalho MG, Gomes MSR, Pereira AHF, Daniel JFS, Schripsema J 2006a. Carbon-13 and proton NMR assignments of a new agathisflavone derivative. *Magn Reson Chem 44*: 35-37.

Carvalho MG, Jr JGR, Cavatti LC, Suzart LR, Cornelius MRF, Silva VC, Cardozo MAR. 2006b. Determinação estrutural de flavonóides com análise de espectros de RMN de ^{1}H 1D e reação com diazometano. *Rev Univ Rural, Ser Ci Exatas e da Terra 25*: 1/2, 46-60.

Daniel JFS, Alves CCF, Grivicich I, Rocha AB, Carvalho MG 2007. Antitumor activity of flavonoids from *Ouratea* and *Luxemburgia* on human cancer cell lines. *Indian J Pharmacol 39*: 184-186.

Likhitwitayawuid K, Rungserichai R, Ruangrungsi NR, Phadungcharoen T 2001. Flavonoids from *Ochna integerrima*. *Phytochemistry 56*: 353-357.

Oliveira MCC, Carvalho MG, Grynberg NF, Brioso PST 2005. Biflavonoid from *Luxemburgia nobilis* as inhibitor of topoisomerases. *Planta Med 71*: 563-566.