J. Braz. Chem. Soc., Vol. 11, No. 2, 143-147, 2000.
Printed in Brazil

0103 - 5053 $6.00+0.00

Article

Chemical Constituents from *Ouratea floribunda*: Complete ^{1}H and ^{13}C NMR Assignments of Atranorin and its New Acetyl Derivative

Mário G. de Carvalho[a*], Geizi J. A. de Carvalho[a] and Raimundo Braz-Filho[b]

[a]*Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, 23851-970, Seropédica - RJ, Brazil*

[b]*Setor de Química de Produtos Naturais-LCQUI- CCT, Universidade Estadual do Norte Fluminense, 28015-620, Campos - RJ, Brazil.*

O fracionamento cromatográfico do extrato hexânico da madeira de *Ouratea floribunda* (Ochnaceae) forneceu, além dos triterpenos friedelina (**1**), friedelanol (**2**) e lupeol (**3**), o depsídeo atranorina (**4**). As estruturas das substâncias isoladas foram determinadas através da análise dos dados espectroscópicos de RMN ^{1}H e ^{13}C, massas e comparação com dados da literatura. A atribuição inequívoca dos deslocamentos químicos dos átomos de carbono e hidrogênio do depsídeo **4** e do seu novo derivado monoacetilado **4a** foi realizada através de técnicas uni- e bidimensionais de RMN (^{1}H-^{1}H COSY e NOESY, ^{1}H-^{13}C HMBC e HMQC) e está sendo descrita pela primeira vez na literatura.

Chromatographic fractionation of the hexane extract from the wood of *Ouratea floribunda* (Ochnaceae) afforded friedelin (**1**), friedelanol (**2**), lupeol (**3**) and the depside atranorin (**4**). The structure elucidation of the isolated compounds was performed by spectrometric analysis involving comparison with literature data. The unambiguous assignments of ^{1}H and ^{13}C NMR data of atranorin **4** and its acetyl derivative **4a** are reported for the first time and involved ^{1}H-^{1}H homonuclear (COSY and NOESY) and ^{1}H-^{13}C heteronuclear (HMQC and HMBC) NMR experiments.

Keywords: *Ouratea floribunda, Ochnaceae*, atranorin, monoacetylatranorin

Introduction

The genus *Ouratea* (Ochnaceae) comprises *ca* 300 tropical species occurring mainly in South America[1]. Some species of this genus have been shown to possess antiviral (*Ouratea lucens*, from Panama)[2], antimicrobial (*O. parviflora*, from Brazil)[3] as well as pain relief (*O. reticulata*, from Guinea) activities[4]. The species *O. spectabilis*, from Brazil, has been reported to be used in folk medicine for the treatment of rheumatic and gastric distress. Recently, the inhibition of bovine lens aldose reductase by a biflavone isolated from this species has been described[5].

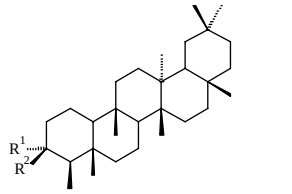
The chemical study of this genus has led to the isolation of a biscatequin (*Ouratea* sp.)[6], a cyanidin and a flavonoid (*O. affinis*)[7], biisoflavanones, an isoflavone and triterpenes (*O. hexasperma*)[8], biflavones (*O. spectabilis*)[5] and recently we reported the isolation of biflavones, flavonoid glycosides, chloroisoflavonoids[9], norisoprenoids, lignans, a diterpene[10], triterpenes and steroids from *O. semiserrata*[11].

As part of our chemical and pharmacological studies of Brazilian plants we reported the investigation of some *Ouratea* species[8-11]. In those studies, we also detected the inhibition of murine tumour growth and an antiproliferative effects and activation of apoptosis on Ehrlich tumour cells by biflavones isolated from two species belonging to this genus[12].

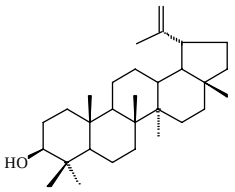
In this first study of *O. floribunda* we report the presence of known triterpenes (**1 - 3**) and describe the spectroscopic data of the depside **4** and of its new monoacetyl derivative **4a**. The structure elucidation of the isolated compounds was performed by spectrometric analysis involving comparison with literature data. The unambiguous assignments of ^{1}H and ^{13}C NMR data of atranorin **4** and of its monoacetyl derivative **4a** are reported for the first time and involved ^{1}H-^{1}H homonuclear (COSY and NOESY) and ^{1}H-^{13}C heteronuclear (HMQC and HMBC) NMR experiments.

Results and Discussion

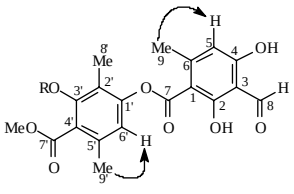
The hexane extract of the dry wood of *Ouratea floribunda* (St. Hill) Eng. was submitted to chromatography on a



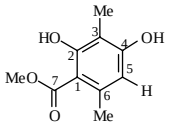
1 R^1, R^2=O
2 R^1=OH, R^2=H



3



4 R=H
4a R=Ac
(⟶ observed NOE)



4b

silica gel column to afford three triterpenes **1 - 3** (3.4 x 10^{-2} % of dry wood weight) and the depside **4** (9.0 x 10^{-4} % of dry wood weight). The known triterpenes friedelin (**1**), friedelanol (**2**) and lupeol (**3**) were identified by their ^{1}H and ^{13}C NMR spectral data analysis and comparison with literature values[13-15].

The molecular formula of **4** was deduced as $C_{19}H_{18}O_8$ by a combination of LRMS (*m/z* 375 $[MH]^+$, 15%, $C_{19}H_{19}O_8$), ^{1}H and ^{13}C (HBBD and DEPT) NMR spectra analysis. Its ^{1}H NMR spectrum showed the presence of singlet signals at δ_H 2.07, 2.53, 2.67 and 3.97 corresponding to three methyl groups bonded to sp^2 carbons and one methoxyl group, respectively. This spectrum also revealed six deshielded singlet signals corresponding to two aromatic hydrogens (δ_H 6.39 and 6.50), a formyl hydrogen (δ_H 10.30) and three quelated hydroxyl groups (δ_H 11.94, 12.49 and 12.54). The comparative analysis of HBBD, DEPT and HMQC NMR spectra (Table 1) showed twelve quaternary sp^2 carbon signals, three CH (δ_C 112.8, 115.9 and 193.8) and four CH_3 (δ_C 9.4, 24.0, 25.6 and 52.3) consistent with the data obtained by ^{1}H NMR analysis. The chemical shifts of quaternary sp^2 carbons [δ_C 102.8, 108.8, 110.3, 116.7, 139.9, 152.0, 152.4, 162.8, 167.5, 169.1, 169.7 (C=O), and 172.2 (C=O)] were used to propose two aromatic rings sustaining five substituents (*vide infra*) and one hydrogen atom [δ_H 6.50 (s) and 6.39 (s)] each.

The HMBC experiments showed $^{2,3}J_{CH}$ long-range correlations (Table 1) between the 3H-9' (δ_H 2.53) and carbons C-5' (δ_C 139.9, ^2JCH), CH-6' (δ_C 115.9, $^3J_{CH}$) and C-4' (δ_C 110.3, $^3J_{CH}$); between 3H-8' (δ_H 2.07) and C-2' (δ_C 116.7, $^2J_{CH}$), C-3' (δ_C 162.8, $^3J_{CH}$) and C-1' (δ_C 152.0, $^3J_{CH}$); and between H-6' (δ_H 6.50) and C-1' (δ_C 152.0, $^2J_{CH}$), (C-2' (δ_C 116.7, $^3J_{CH}$) and C-4' (δ_C 110.3, $^3J_{CH}$). Therefore, the two methyl groups CH_3-8' and CH_3-9' are at the same ring. The other cross peaks (Table 1) are also in agreement with the location of one methoxycarbonyl and one hydroxyl group in this same aromatic ring. These deductions and

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of atranorin (**4**) and its acetyl derivative **4a**, in $CDCl_3$ and TMS as internal standard.

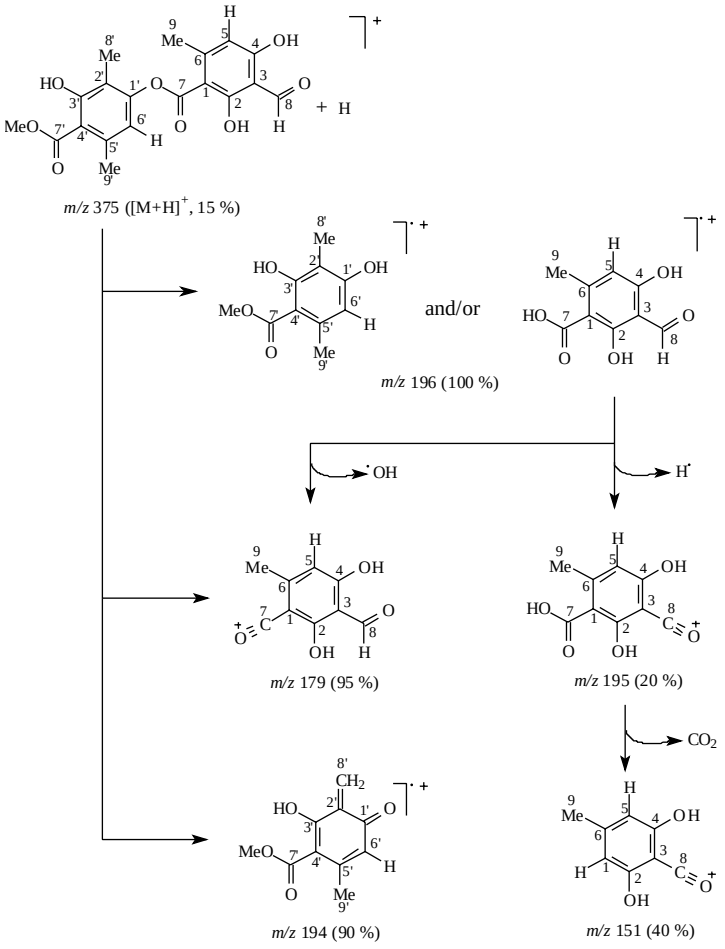
	4			**4a**		
C/H	$\delta_C(CH_X)^{a,b}$	$\delta_H(n_H, m)^a$	HMBC[c]	$\delta_C(CH_X)^{a,b}$	$\delta_H(n_H, m)^a$	HMBC[c]
1	102.8(C)	-	HO-2; H-5,9	102.7(C)	-	H-5
2	169.1(C)	-	HO-2	169.1(C)	-	
3	108.5(C)	-	HO-2,4; H-5	108.6(C)	-	HO-2,4; H-5
4	167.5(C)	-	H-5; HO-4	167.6(C)	-	HO-4
5	112.8(CH)	6.39 (1, s)	HO-4; H-9	112.9(CH)	6.39(1, s)	H-9
6	152.4(C)	-	H-9	152.4(C)	-	H-9
7	169.7(C)	-	-	168.4(C)	-	-
8	193.8(CH)	10.30 (1, s)	-	193.8(CH)	10.40 (1, s)	-
9	25.6(CH_3)	2.67 (3, s)	H-5	25.6(CH_3)	2.66 (1, s)	
1'	152.0(C)	-	H-6'; H-8'	149.7(C)	-	H-6'; H-8'
2'	116.7(C)	-	HO-3'; H-6',8'	118.3(C)	-	H-8'
3'	162.8(C)	-	HO-3'; H-8'	148.4(C)	-	H-8'
4'	110.3(C)	-	HO-3'; H-6',9'	124.5(C)	-	H-6'
5'	139.9(C)	-	H-9'	136.6(C)	-	H-9'
6'	115.9(CH)	6.50 (1, s)	H-9'	121.8(CH)	6.91 (1, s)	H-9'
7'	172.2(C)	-	H_3CO-7'	166.5(C)	-	H_3CO-7'
8'	9.4(CH_3)	2.07 (3, s)	-	10.3(CH_3)	2.01 (3, s)	-
9'	24.0(CH_3)	2.53 (3, s)	H-6'	20.1(CH_3)	2.66 (3, s)	H-6'
H_3C-O	52.3(CH_3)	3.97 (3, s)	-	52.3(CH_3)	3.89 (3, s)	-
H_3C=O	-	-	-	169.7(C)	-	H_3C-C=O
H_3C-O	-	-	-	20.7(CH_3)	2.30 (3, s)	
HO-2	-	12.49 (1, s)	-	-	12.44 (1, s)	-
HO-4	-	12.54 (1, s)	-	-	12.53 (1, s)	-
HO-3'	-	11.94 (1, s)	-	-	-	-

[a]Number of hydrogens bonded to carbons were deduced by comparative analysis of HBBD and DEPT ^{13}C NMR experiments; [b]Assignments aided by NOESY and HMQC experiments; [c]HMBC J_{CH} value = 9 Hz.

the peaks at *m/z* 196 (100 %), 194 (90 %) and 179 (95 %) observed in the mass spectrum (Scheme 1) are in agreement with the presence of a methyl 2-hydroxy-3,6-dimethylbenzoate moiety. The nuclear Overhauser enhancements observed in ^{1}H-^{1}H-NOESY spectrum was used to confirm the location of 3H-9' (δ_H 2.53) ortho to H-6' (δ_H 6.50). The remaining $^{2,3}J_{CH}$ cross peaks observed in the HMBC spectrum were used to establish the correlation between the signals of two hydroxyl, HO-2 (δ_H 12.49) with C-2 (δ_C 169.1, $^2J_{CH}$) and C-3 (δ_C 108.5, $^3J_{CH}$), HO-4 (δ_H 12.54) with C-3 (δ_C 108.5, $^3J_{CH}$), C-4 (δ_C 167.5, $^2J_{CH}$) and CH-5 (δ_C 112.8, $^3J_{CH}$), and to assign the chemical shifts for C-2, C-3, C-4 and C-5 (Table 1). The observed long-range correlation involving the remaining methyl group CH_3-9 (δ_C 25.6) and the aromatic hydrogen H-5 (δ_H 6.39) led to the assignments of C-1 and C-6 (Table 1). The NOE observed between H-5 (δ_H 6.39) and 3H-9 (δ_H 2.67) in ^{1}H-^{1}H NOESY experiment was also used to confirm the location of 3H-9 ortho to H-5. These data and the peaks at *m/z* 196 (100 %),195 (20%), 179 (95 %) and 151 (40 %) observed in the mass spectrum (Scheme 1) are in accordance with the presence of a 2,4-dihydroxy-3-aldehyde-6-methyl-benzoate moiety. Thus, these data allowed to deduce the structure of **4**, a natural product classified as depside and known as atranorin, isolated from lichens. The structure was previously determined only by ^{1}H NMR data and confirmed through synthesis[16-18].

The acetylation of **4** at 60°C yielded the hydrolysis product, methyl 2-hydroxy-4-hydroxy-3,6-dimethylbenzoate (**4b**). The same acetylation carried out at room temperature gave the new monoacetate **4a**, whose structure was confirmed by complete ^{1}H and ^{13}C NMR spectra assignment (Table 1). As was expected, the chemical shift of H-6' was shifted from δ_H 6.50 in **4** to δ_H 6.91 in **4a**, with its location at *ortho* or at *para* positions and, in accordance with the presence of H-6' *para* in relation to the 3'-hydroxyl group in 4[19].

This is the first report for ^{13}C NMR data assignments for the depside **4** [3-formyl-2,4-dihydroxy-6-methylbenzoic acid 3-hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenyl ester] and the derivative **4a** [3-formyl-2,4-dihydroxy-6-methylbenzoic acid 3-acetoxy-4-(methoxycarbonyl)-2,5-dimethylphenyl ester].



Scheme 1. Proposed fragmentation mechanisms of **4** (only peaks classified as principal).

Depsides are aromatic products of polyketide origin which are especially well represented in lichens, the most characteristic being formed by the condensation of two or three simple orcinol or β-orcinol-type phenolic moieties linked by an ester bond. A large number of structurally related metabolites have been detected in several lichens. Although recently a tridepside fenviorin was isolated from the plant *Frullania nisqualensis*[20]. Atranorin is a *para*-β-orcinol depside bearing CO_2Me, CHO and OH groups, frequently found in lichens, it seems that the wood of *O. floribunda* used in this study was contaminated. Among the aromatic lichen compounds, atranorin is the most frequent allergenic agent responsible for airborne contact dermatitis[21,22].

Experimental

General experimental procedures

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The NMR spectra of compounds **1 - 3** were taken on a Bruker AC 200 (^{1}H: 200 MHz; ^{13}C: 50.3 MHz) spectrometer. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR (1D and 2D) spectra of **4** and its acetyl derivative **4a** were recorded on a Varian UN 400 spectrometer. HMQC and HMBC experiments were optimized for $^1J_{CH}$ = 140 Hz and $^nJ_{CH}$ (n=2 and 3) = 9 Hz, respectively. Mass spectra were obtained with a VG Quattro instrument. FTIR spectra were recorded as a film on a Perkin-Elmer 1500 spectrometer. Column chromatography and on TLC plates were performed using silica gel Merck.

Plant material

Ouratea floribunda (St. Hill) Engl. was collected at an environmentally preserved area near Centro de Pesquisas Morro do Chapéu, Belo Horizonte, Minas Gerais, Brazil, in 1996. Identification of the plant material was performed by Jorge L. Silva by comparison with a herbarium specimen (nº 6944) deposited at the Herbarium José Badini in the Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Minas Gerais State, Brazil.

Extraction and isolation of the constituents

Dried wood material (1334 g) was powdered and sequentially extracted with hexane, EtOAc and MeOH by maceration at room temperature. The hexane extract was evaporated under vacuum to yield 4.3 g of residue. This residue was subjected to chromatography on silica gel column using hexane, dichloromethane and methanol with a gradient of polarity from dichloromethane to methanol. Thirty three fractions of 125 mL each were collected. Fractions 1-5 yielded a mixture of **1** and **2** (120 mg, m. p. 248-250 ºC). Fractions 9-10 yielded **3** (330 mg, m. p. 160-161ºC). Fractions 11-14 gave **4** (12 mg, m. p. 191-192 ºC).

Atranorin (**4**)

Colorless crystals from hexane, m. p. 191-192 ºC. IR (KBr) ν_{max} (cm^{-1}): 3450, 1770, 1730, 1650, 1580, 1260, 1145. ^{1}H (400 MHz, $CDCI_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR: Table 1. CIMS: Scheme 1.

Acetylation of atranorin (**4**)

Compound **4** (9 mg) was dissolved in pyridine (1.0 mL) and acetic anhydride (1.0 mL), and the solution was allowed to stand for 24 h at room temperature. Usual work-up yielded **4a** (gum, 7 mg), amorphous powder from hexane, m. p. 105-106 ºC, IR (film) ν_{max} (cm^{-1}): 1772, 1731, 1649, 1572, 1260, 1150. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCI_3$): Table 1. CIMS *m/z* (rel. int.): 417 ([M + H]$^+$, 2), 239 (20), 221 (25), 207 (100), 197 (73), 179 (62).

The acetylation of **4** at 60 ºC yielded methyl 2-acetoxy-4-hydroxy-3,6-dimethylbenzoate (**4b**): gum, ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 6.52 (s, H-5), 3.83 (s, MeO-7), 2.30 (s, 3H), 1.99 (s, 3H).

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research fellowships and for grants to G. J. A. de C.. We thank Mr. J. L. Silva and Dr. A. A. Werle (Universidade Federal de Ouro Preto, Minas Gerais, Brazil) for the collection and identification of plant material. We thank Dr D. G. I. Kingston, Virginia Polytechnic Institute and State University-USA, for mass, ^{1}H and ^{13}C NMR spectrometers facilities and Dr. Victor Runjanek for reading the manuscript.

References

1. Heywood, V. H. In *Flowering Plants of the World*; Oxford University Press; London, **1978**, p.82.
2. Roming, T. L.; Weber, N. D.; Murray, B. K.; North, J. A.; Wood, J. C.; Hughs, A. G.; Cates, R. G. *Phytother. Res*. **1992**, *6*, 38.
3. Paulo, M. Q.; Lima, E. O.; Maia, R. F.; Filho, X. L. *Chin Chiao I Sheng Tsa Chin* **1986**, *83*, 19.
4. Vasileva, B. *Plantes Medicinales De Guine*; Conakry, Rep. De Guine, Moscow, Univ. Moscow-USSK, 1969.
5. Felicio, J. D.; Gonçalvez, E.; Braggio, M. M.; Costantino, L.; Albasini, A.; Lins, A. P. *Planta Med*. **1995**, *61*, 217.

6. Oliveira, M. M. de; Sampaio, M. P.; Simon, F.; Gilbert, B.; Mors, W. B. An. *Acad. Bras. Cienc.* **1972**, *44*, 42.
7. Key, M. C.; Waterman, P. G. *Biochem. Syst. Ecol.* **1980**, *8*, 401.
8. Moreira, I. C.; Sobrinho, D. C.; Carvalho, M. G. de; Braz-Filho, R. *Phytochemistry* **1994**, *35*, 1567.
9. Velandia, J. R.; Carvalho, M. G. de; Braz-Filho, R. *Nat. Prod. Letters* **1998**, *12*, 191.
10. Velandia, J. R.; Carvalho, M. G. de; Braz-Filho, R. *Quím. Nova* **1998**, *21*, 397.
11. Velandia, J. R. *Constituintes quimicos de* Ouratea semiserrata *e transformações químicas da neolignana aureina*; Tese de Doutoramento; CPGQO- Departamento de Química, Univ. Fed. Rural do Rio de Janeiro, Seropédica, RJ., 1997.
12. a) Grynberg, N. F.; Mortorelli, R. A.; Carvalho, M. G. de; Braz-Filho, R.; Moreira, I. C.; Santos, A. C. S.; Echevarria, A. *Proceedings of the XVI International Cancer Congress*; Monduzzi ed.; Bologna, 1994, p 63. b) Grynberg, N. F.; Brioso, P. S. T.; Velandia, J. R.; Echevarria, A.; Carvalho, M. G. de; Braz-Filho, R. *Proceedings of the XVIl lnternational Cancer Congress*, Monduzzi ed.; Bologna, 1998, p 317.
13. Carvalho, M. G. de; Almeida, M. E. L. de; Hauptli, M. B.; Meleiro, L. A. C. *Rev. Univ. Rural, Ser. Cienc. Exatas e da Terra* **1995**, *17*, 33.
14. Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517.
15. Olea, R. S. C.; Roque, N. F. *Quím. Nova* **1990**, *13*, 171.
16. Huneck, S.; Linscheid, P. *Z. Naturforsch.* **1968**, *23b*, 717.
17. Elix, J. A.; Mahadevan, I.; Wardlaw, J. H.; Arvidson, L.; Jorgensen, P. M. *Aust. J. Chem.* **1987**, *40*, 1581.
18. Alix, J. A.; Barclay, C. E.; David, F.; Griffin, F. K.; Hill, A. M.; McConnel, D. B.; Wardlaw, J. H. *Aust. J. Chem.* **1993**, *46*, 301.
19. Carvalho, M. G. de; Braz-Filho, R. *Quím. Nova* **1993**, *16*, 89.
20. King, Y. C.; Bolzani, V. da S.; Baj, N.; Gunatilaka, A. A. L.; Kingston, D. G. I. *Planta Med.* **1996**, *62*, 61.
21. Sandberg, M.; Thune, P. *Contact Dermatitis* **1984**, *11*, 168.
22. Lorenzi, S.; Guerra, L.; Vezzani, C.; Vincenzi, C. *Contact Dermatitis* **1995**, *32*, 315.

Received: June 1, 1998