New *nor*-Cucurbitacin Glucosides from *Wilbrandia* Species

Maria Elisa O. Matos

Departamento de Farmácia, Centro de Ciências da Saúde,
Universidade Federal do Ceará, Rua Capitão Francisco Pedro, 1210
60415-970 Fortaleza, Ceará, Brasil.

Maria Iracema L. Machado, Francisco José A. Matos

Departamento de Química Orgânica e Inorgânica
Universidade Federal do Ceará, Caixa Postal 12200,
60451-970 Fortaleza, Ceará, Brasil.

and

Raimundo Braz Filho

Departamento de Química, Instituto de Ciências Exatas,
Universidade Federal Rural do Rio de Janeiro, Caixa Postal 74541
23851-970 Seropédica, Rio de Janeiro, Brasil

Received: september 9, 1993; november 22, 1993.

Duas novas *nor*-cucurbitacinas glicosídicas (**3** e **4**) foram isoladas das raizes de um espécime de *Wilbrandia* sp e suas estruturas foram elucidadas com base na análise de espectros de RMN uni- (1D) e bidimensionais (2D), em comparação com os dados das cucurbitacinas **1** e **2** isoladas da mesma espécie.

Two new *nor*-cucurbitacin glucosides (**3** and **4**) were isolated from the roots of a specimen of *Wilbrandia* sp. Their structures were elucidated by 1D and 2D NMR analysis, and comparison with the cucurbitacins **1** and **2** earlier isolated from the same source.

Key words: Wilbrandia *sp; roots; Cucurbitaceae;* nor-*cucurbitacin glucosides; spectral data.*

Introduction

We have previously reported[1] the isolation of two *nor*-cucurbitacin glucosides (**1** and **2**) from *Wilbrandia* species which were converted into their peracetyl derivatives **1a** and **2a** for separation and identification. In view of the medicinal potencial of these species[2], we have now re-examined a larger amount of *Wilbrandia* sp extract that afforded again **1** and **2** in a mixture with two new *nor*-cucurbitacin glucosides **3** and **4**. The structure of compound 4 was elucidated on the basis of spectral data of its peracetyl derivative **4a**. 1D and 2D NMR analyses were performed and the new structures were correlated with the published NMR spectra of similar compounds.[3,4]

Results and Discussion

In this paper we describe the isolation and identification of two new triterpenes **3** and **4** in mixtures with **1** and **2**, respectively, from the roots of a specimen of *Wilbrandia* sp.

The identification of cucurbitacin glucoside **1**, in the mixture of **1** and **3**, as 6,7-dehydrofevicordin A glucoside, a glucoside previously isolated from this plant,[1] allowed to recognize the presence of the additional signals in the ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra consistent with the modification of the double bond localized between carbon atoms C-23 [**1**: δ_H 6.67 (d,J=15.1 Hz); δ_C 122.53 (d)] and C-24 (**1**: δ_H 7.06 (d,J=15.1 Hz); δ_C 151.61 (d)] to the corresponding 23,24-dihydroderivative (**3**: δ_H 2.7-2.5 (m, 2H-23), 1.4-1.1 (m, 2H-24); δ_C 32.78 (t, C-23) and 35.73 (t, C-24)].

Table 1. *^{1}H NMR (200 MHz) data of compounds **1** and **3** (CD_3OD + $CDCl_3$) and peracetyl derivatives **2a** and **4a** ($CDCl_3$), TMS as internal standard. The chemical shifts are expressed in δ (ppm) and the coupling constants (J) in Hz (in parenthesis).*

H	1	1 + 3	3	2a	2a + 4a	4a
1		6.48(s)			6.45(s)	
6		6.79(d,10.1)			6.80(d,10.0)	
7		5.72(dd,10.1,5.8)			5.78(dd,10.0,6.4)	
8		2.46(d,5.8)			2.64(d,6.4)	
12		3.0-2.8			2.89(d,16.4)	
		2.7-2.5			2.77(d,16.4)	
15		2.0-1.8			a	
		1.3-1.1			a	
16		4.5-4.3			5.50(t,7.0)	
17		2.37(d,6.9)			2.57(d,7.1)	
18	0.88(s)		0.86(s)		0.91(s)	
19		1.29(s)			1.29(s)	
21		1.45(s)		1.47(s)		1.32(s)
22	-	-	-	-		5.00(d,7.1)
23	6.67(d,15.1)		2.7-2.5			5.62(dd,14.3,7.1)
24	7.06(d,15.1)		1.4-1.1			5.86(d,14.3)
26		1.34(s)			1.25(s)b	
27		1.31(s)			1.23(s)b	
28		2.14(s)			2.27(s)	
30		1.14(s)			1.09(s)	
1'		4.54(d,7.2)			4.80(d,7.1)	
2'		3.7-3.3			5.3-5.1	
3'		3.7-3.3			5.3-5.1	
4'		3.7-3.3			5.3-5.1	
5'		3.7-3.3			3.87(m)	
6'		3.90(dd,12.7,2.3)			4.30(dd,11.0,3.7)	
		3.76(dd,12.7,3.7)			4.23(dd,11.0,3.2)	
OAc	1.91(s)		1.85(s)		-	
					2.17(s)	
					2.13(s)	
					2.09(s)	
					2.07(s)	
					2.05(s)	
					2.04(s, 2x)	
					2.03(s)	
					1.94(s)	

[a] *Overlapped with other signals.*

This modification was confirmed by the downfield shift ($\Delta\delta_C$=215.52-204.39=11.13 ppm) observed in the C-22 signal (δ 204.39) of **1** compared with the chemical shift of this carbon atom (δ_C 215.52) in **3**. As anticipated, this chemical shift alteration was also confirmed by reduction [**3**: δ_C 32.78 (t, C-23), 35.73 (t, C-24)] of the double bond [**1**: δ_C 122.53 (d, C-23), 151.61 (d, C-24)] of the conjugated carbonyl system. The remaining data obtained from the ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra was considered practically identical, since the less significant modifications observed in the chemical shifts of C-21 and C-25 were also used as additional support for the deduction described above. Thus, all the spectral data (Tables 1 and 2) of the new *nor*-cucurbitacin glucoside present in the mixture with **1** allowed its characterization as 23,24-dihydro-6,7-dehydrofevicordin A glucoside (**3**).

The mixture containing the *nor*-cucurbitacins **2** and **4** was transformed to peracetyl derivatives **2a** and **4a** by the usual treatment with acetic anhydride-pyridine. The analysis of the ^{1}H (Table 1) and ^{13}C NMR spectra (Table 2) revealed that the two peracetyl derivatives differ significantly only in the chemical shifts of the side-chain carbons at C-17. The remaining signals of ^{1}H and ^{13}C corresponding to this side-chain, after assignment of the absorptions belonging to **2a**, were assigned to the isomer **4a**, containing one hydroxy group at C-22 [δ_H 5.00 (d,J=7.1 Hz, H-22); δ_C 77.20 (d, C-22)] and one **E** double bond (J=14.3 Hz) localized between C-23 and C-24 [δ_H 5.62 (dd,J=14.3 and 7.1 Hz, H-23) and 5.86 (d,J=14.3 Hz, H-24); δ_C 120.20 (d, C-23) and 144.67 (d, C-24)] (Tables 1 and 2). The assignments of the signals of these protons were ascertained by the cross peaks in the homonuclear shift correlated 2D NMR (^{1}Hx^{1}H-COSY) spec-

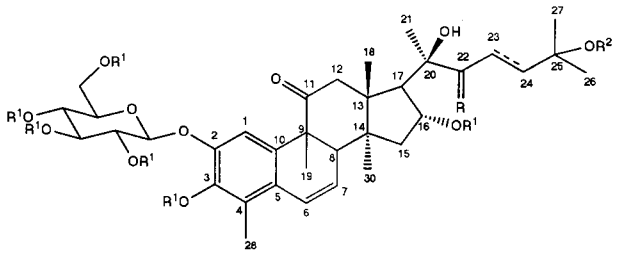
Table 2. *^{13}C NMR (50.3 MHz) data of compounds **1** and **3** (CD_3OD+$CDCl_3$) and peracetyl derivatives **2a** and **4a** ($CDCl_3$), TMS as internal standard. The chemical shifts are expressed in δ (ppm) and the multiplicity of the signals deduced by DEPT technique.*

C	1	1+3	3	2a	2a+4a	4a
1	112.53		112.40		108.38	
2	145.65		145.14		147.16	
3		129.68			137.00	
4		123.23			128.25	
5		128.17			126.19	
6		127.21			125.93	
7		126.09		124.66		124.45
8		46.12		45.98		46.00
9		50.41		50.51		50.74
10		129.68			135.04	
11	215.95		215.89a		213.16	
12	52.05		51.89	51.12		51.41
13		48.85			47.63	
14		50.41			48.57	
15	44.74		44.64	41.23		41.89
16	71.77		72.13		74.64	
17	60.45		59.85	54.15		52.89
18		20.33			19.54	
19		18.05			17.08	
20	80.15		80.75	78.57		74.22
21	25.39		26.20	24.19		26.29
22	204.39		215.52a	213.53		77.20
23	122.53		32.78	30.66		120.20
24	151.64		35.73	37.03		144.67
25	83.07		81.01		70.30	
26	26.63		26.74	29.34		29.83
27	26.63		26.29	29.34		29.83
28		11.28			11.77	
30	21.88		20.56		21.57-20.19	
1'		104.62			97.88	
2'		74.71			70.29	
3'		78.06			72.53	
4'		70.75			67.87	
5'		77.59			72.07	
6'		61.96			61.61	
OAc		20.54			21.38	
		20.32			21.10	
	172.45		171.89		20.59	
					20.51(2x)	
					20.19	
					171.11	
					170.90	
					170.15	
					169.43	
					169.36	
					168.52	

[a] *Signals interchangeable*

trum, along with other spin-spin interaction of protons in the molecules of two components of the mixture of **2a** and **4a** (Tables 1 and 2). Thus, the structure of the peracetyl derivative was established as **4a** and, consequently, the structure of the additional new *nor*-cucurbitacin glucoside isolated from *Wilbrandia* sp was defined as 22-dihydro-25-O-deacetyl-6,7-dehydrofevicordin A glucoside (**4**).

The assignments of the ^1H (Table 1) and ^{13}C (Table 2) chemical shifts of **3** and **4a** were based on the application of the usual chemical shift parameters, the observed multiplicity



	R	R^1	R^2	
1	O	H	Ac	Δ^{23}
1a	O	Ac	Ac	Δ^{23}
2	O	H	H	23,24-dihydro
2a	O	Ac	H	23,24-dihydro
3	O	H	Ac	23,24-dihydro
3a	O	Ac	Ac	23,24-dihydro
4	H,OH	H	H	Δ^{23}
4a	H,OAc	Ac	H	Δ^{23}

of ^1H absorptions and ^{13}C signals in the DEPT-^{13}C NMR (number of attached protons for each carbon signal) spectra, the homonuclear 2D shift-correlated ($^1Hx^1H$-COSY) NMR spectrum[5] (**4a**) and comparison with the corresponding values of the *nor*-cucurbitacin glucoside **1** and of the peracetyl derivative (**2a**) of **2**, isolated previously from the same *Wilbrandia* sp.[1]

Experimental

General experimental procedures. M.p. were determined using a Kofler hot stage instrument and are not corrected. IR spectra were taken on a Perkin Elmer 257 spectrometer. ^1H and ^{13}C NMR spectra were recorded in CD_3OD+$CDCl_3$ (**1** + **3**) and $CDCl_3$ (**2a** + **4a**), using TMS as internal standard employing a Bruker AC 200 (^1H: 200 MHz; ^{13}C: 50.3 MHz) spectrometer.

Plant material. Roots of *Wilbrandia* sp. were colected at Senador Pompeu, Ceará State, Brazil. A voucher specimen (N° 13623) is deposited in the Herbarium Prisco Bezerra, Instituto de Biologia, Universidade Federal do Ceará, Fortaleza.

Extraction and isolation. Plant material (4 kg) was extracted with EtOH in a Soxhlet apparatus as previously described.[1] The residue (200 g), obtained after concentration of the extract under reduced pressure, was chromatographed on a silica gel (600 g) column. Elution with EtOAc-MeOH (4:1) furnished fraction A (18 g) and elution with MeOH gave fraction B (30 g). Fraction A (3 g) was rechromatographed on a silica gel (20 g) column and eluted with mixtures of increasing polarity of hexane and EtOAc. The fraction eluted with hexane-EtOAc (1:9) yielded the mixture of **1** and **3** (250 mg). The fraction B (4 g) was acetylated with Ac_2O (30 ml) and pyridine (10 ml) and chromatographed (4.5 g) on a silica gel column (30 g). The fraction eluted with hexane-EtOAc (1:3) yielded the mixture of **2a** and **4a** (150 mg).

Mixture of **1** *and* **3**. Powder, m.p. 155-158 °C; IR (KBr), cm^{-1}: 3400 (OH), 1720-1680 (11,22-di*oxo*), 1620-1560 (aromatic); ^1H NMR: see Table 1; ^{13}C NMR: see Table 2.

Mixture of **2a** *and* **4a** - Powder, m.p. 122-125 °C; IR (KBr), cm^{-1}: 3400 (OH), 1755 (esters), 1700 (11,22-di*oxo*), 1600, 1500 (aromatic); ^1H NMR: see Table 1; ^{13}C NMR: see Table 2.

Acknowledgements

This work was supported by CNPq research fellowships and by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) and Financiadora de Estudos e Projetos (FINEP). The authors are grateful to Professors Edilberto R. Silveira and José A. Pinheiro for the acquisition of the ^1H and ^{13}C NMR spectra at the Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará

References

1. M. Elisa O. Matos, M. Iracema L. Machado, A. A. Craveiro, F. J. A. Matos, R. Braz-Filho, *Phytochemistry* **30**, 1020 (1991).
2. F.R.C. Almeida, V.S.N. Rao, M. Elisa O. Matos, *Brazil. J. Med. Biol. Res.* **22**, 1397 (1989).
3. R. Bauer, L.H. Berganza, O. Seligmann, H. Wagner, *Phytochemistry* **24**, 1587 (1985).
4. H. Stuppner, H. Kahlig, O. Seligmann, H. Wagner, *Phytochemistry* **29**, 1633 (1990).
5. E. Breitmaier, W. Voelter, *Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry* (3rd edition, VCH, Weinheim, 1987).