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Note

Four known triterpenoids isolated from three Brazilian plants: ^{1}H and ^{13}C chemical shift assignments

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ABSTRACT: An NMR study of 3-*O*-acetylebelin lactone, 3β,19α,23-trihydroxyurs-12-en-28-oic acid, 3-oxoolean-18-en-28-oic acid and 7-oxofriedelin is described. In addition to conventional 1D NMR methods, 2D shift-correlated NMR experiments [$^{1}H \times {}^{1}H$-COSY, $^{1}H \times {}^{13}C$-COSY–$^{1}J_{CH}$ (HETCOR and HMQC), $^{1}H \times {}^{13}C$-COSY–$^{n}J_{CH}$ ($n = 2$ and 3, COLOC and HMBC)] and 2D $^{1}H \times {}^{1}H$-NOESY were used for ^{1}H and ^{13}C chemical shift assignments of these triterpenoids. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: triterpenoids; NMR; 1D NMR; 2D NMR; ^{1}H and ^{13}C chemical shift assignments

INTRODUCTION

This paper reports the ^{1}H and ^{13}C chemical shift assignments of 3-*O*-acetylebelin lactone (**1a**/**1b**), 3β,19α,23-trihydroxyurs-12-en-28-oic acid (**2**), 3-oxoolean-18-en-28-oic acid (**3**) and 7-oxofriedelin (**4**).

The results of the application of 1D and 2D spectral techniques were used to identify the structures and to establish the ^{1}H and ^{13}C resonance assignments of these triterpenes, which were also utilized to confirm ^{13}C NMR data already published for **1**, **2a**, **3a** and **4**.[1,2]

To the best of our knowledge, ^{13}C NMR spectral data of **1a**/**1b** and **3** are hitherto unreported. These data can be utilized in further investigations of natural products.

These triterpenes were isolated during a phytochemical investigation of specimens of the plants *Zizyphus joazeiro* (**1**), *Guettarda platypoda* (**2** and **4**) and *Waltheria viscosissima* (**3**).

EXPERIMENTAL

Plant material and isolation of the constituents

Zizyphus joazeiro Mart. was collected in April 1996 in Piripirituba, *Guettarda platypoda* D.C. in Santa Rita and *Waltheria viscosissima* St. Hil in April 1996 in the Campus of UFPb, Paraiba State, and identified by botanist Maria de Fátima Agra, Universidade Federal da Paraiba (UFPb), João Pessoa, Paraíba, Brazil. Voucher specimens (Agra 3270, Agra 68 and Agra 3446, respectively) are deposited at the Herbarium of UFPb.

The dried and powdered bark (2.6 kg) from *Zizyphus joazeiro* was extracted in a Soxhlet apparatus using $CHCl_3$ followed by MeOH. The residue (230 g) of the MeOH extract, after removal of the solvent under vacuum, was hydrolysed with 10% HCl in H_2O under reflux for 2 h. The precipitate was filtered (28 g) and chromatographed on a silica gel column. The fraction eluted with benzene yielded ebelin lactone (**1**, 30 mg), previously isolated from *Emmenospermum alphitonioides* and *Zizyphys* spp.,[3] *Zizyphus joazeiro* after acid hydrolysis of saponin fraction[4] and permethylated saponin derivative[5] and acid hydrolysis of saponins of *Hovenia dulcis*.[1] The aglycone moiety of these saponins was also characterized in the triterpene glycoside 3β-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-xylopyranosyl (1 → 2)-β-D-xylopyranosyl]-16β,23(*R*):16α,18α-diepoxy-20(*S*)-hydroxydammar-24-ene isolated from the roots of *Centrorema bracteosum*, Leguminosae–Faboideae.[6] The triterpene **1** was acetylated with acetic anhydride in the presence of pyridine to furnish 3-*O*-acetylebelin lactone (**1a**).

The dried and powdered roots (3.0 kg) from *Guettarda platypoda* were extracted in a Soxhlet apparatus using EtOH. The solvent was removed under vacuum to afford 45 g of residue. This residue was subjected to partitioning with EtOH–hexane (7:3) followed by EtOH–$CHCl_3$ (7:3). The $CHCl_3$ fraction (20 g) was chromatographed on a silica gel column eluted with $CHCl_3$ (fractions 18–27) and $CHCl_3$–MeOH (93:7, fractions 82–101) to give **4** (136 mg) and **2** (75 mg), respectively.

The dried powdered roots (2.5 kg) from *Waltheria viscosissima* were extracted in a Soxhlet apparatus using EtOH and the residue (30 g) obtained after removal of the solvent was submitted to partition with EtOH–hexane (8:2) and EtOH–$CHCl_3$ (8:2). Removal of the

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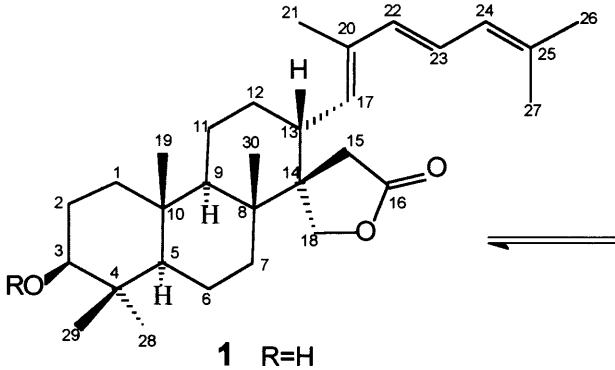

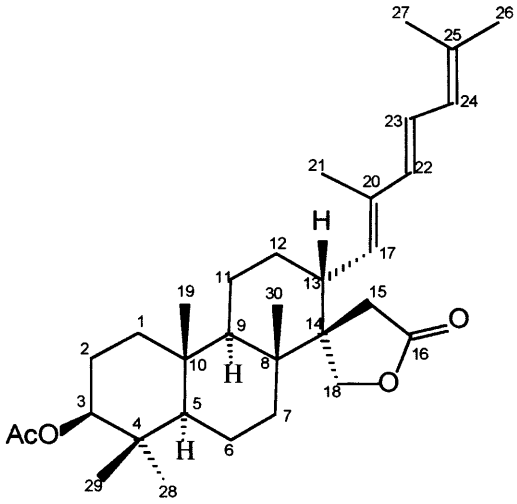

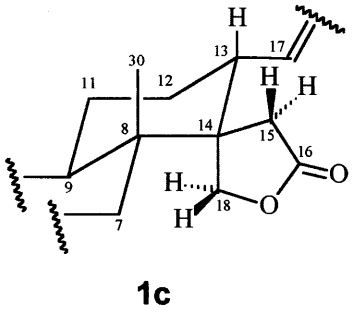

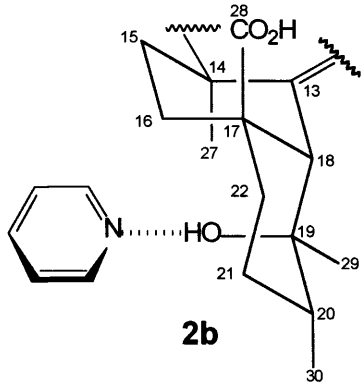

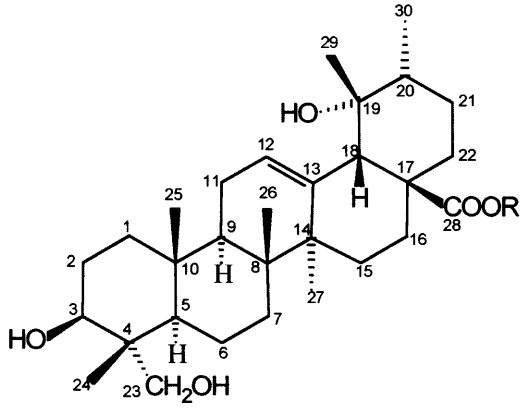

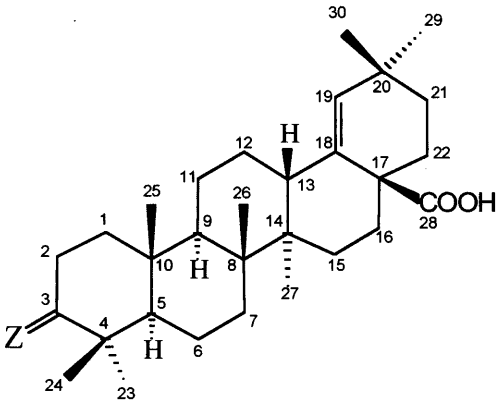

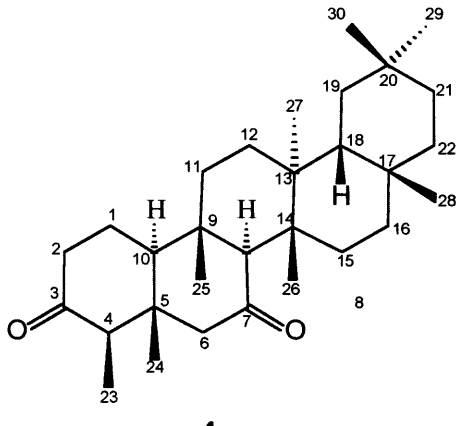

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR for 3-*O*-acetylebelin lactone (**1a**) ($CDCl_3$), including results obtained by heteronuclear 2D shift-correlated HMQC ($^1J_{CH}$) and HMBC ($^nJ_{CH}$, $n = 2$ and 3), in $CDCl_3$ as solvent and residual $CHCl_3$ used as internal reference (δ_H 7.24 and δ_C 77.00), chemical shifts (δ ppm) and coupling constants (J, Hz, in parentheses)[a]

	HMQC		HMBC		
Atom	δ_C[b]	δ_H	$^2J_{CH}$	$^3J_{CH}$	$^1H \times {}^1H$-NOESY
C					
4	37.1 (39.4)	—	H-3, 3H-28 or 3H-29		—
8	40.2 (40.2)	—	3H-30	2H-15, 2H-18	—
10	37.9 (37.3)	—	3H-19		—
14	51.9 (52.0)	—	H-13, 2H-18, 2H-15	3H-30	—
16	77.1 (176.7)	—	2H-15	2H-18	—
20	137.2 (137.0)	—	3H-21, H-22	H-23, H-13	—
25	135.6 (135.2)	—	3H-26, 3H-27	H-23	—
AcO	171.0 (−)	—	CH_3-CO_2	H-3	—
CH					
3	80.6 (77.8)	4.45 (dd, 11.6, 4.8)		3H-28 or 3H-29	H-1α, H-5α, 3H-28
5	55.3 (55.2)	0.80 (m)		3H-28 or 3H-29, H-7	H-3α
9	52.9 (52.7)	0.78 (m)		3H-19, 3H-30	H-18β
13	39.0 (39.5)	2.78 (m)		2H-18, 2H-15	3H-21, 3H-30
17	130.4 (131.6)	5.19 (d, 10.1)	H-13	H-22, 3H-21	H-18α, H-22, H-23
22	134.5 (134.9)	6.07 (d, 15.3)		H-17, H-24, 3H-21	H-17, H-24, 3H-21
23	124.7 (124.7)	6.34 (dd, 10.8, 15.3)	H-22		H-17, H-27
24	125.6 (126.3)	5.82 (d, 10.8)	H-23	H-22, 3H-26 or 3H-27	H-22, 3H-26
CH_2					
1	38.2 (38.7)	1.00, H-1β		3H-19	
		1.67, H-1α			H-3α
2	23.7 (28.0)	1.60	H-3		
6	17.8 (18.1)	1.7–1.4	H-5		
7	34.6 (34.5)	1.49, H-7β		3H-30	H-18β
		1.35, H-7α			
11	20.1 (20.1)	1.6–1.4			
12	29.3 (28.6)	1.14, 1.58			
15	34.3 (35.0)	2.44 (dd, 18.2), H-15β		2H-18	3H-30
		2.08 (d, 18.2), H-15α			
18	69.8 (69.6)	4.37 (d, 10.3), H-18β		H-13, 2H-15	H-7α, H-9α
		4.28 (d, 10.3), H-18α			H-17
CH_3					
19	16.1 (16.2)	0.87 (s)		H-5	3H-30
21	13.4 (13.3)	1.79 (d, 0.9)		H-17, H-22	H-13β, H-22
26	26.3 (26.1)	1.77 (br s)		H-24, 3H-27	H-24
27	18.6 (18.3)	1.76 (br s)		H-24, 3H-26	H-23
28	28.1 (28.6)	0.86 (s)		H-3, 3H-29	
29	16.6 (16.2)	0.85 (s)		H-3, 3H-28	
30	18.0 (18.3)	1.03 (s)			H-15β, H-13β, 3H-19
AcO	21.4 (−)	2.03 (s)			

[a] Homonuclear $^1H \times {}^1H$-COSY spectrum was also used for these assignments. Chemical shifts and coupling constants of hydrogen atoms obtained from 1D ^{1}H NMR spectrum. Superimposed ^{1}H signals are described without multiplicity.
[b] Values (in parentheses) reported in the literature for **1** (pyridine-d_5).[5]

solvent of the $CHCl_3$ solution and column chromatography with a silica gel column eluted with $CHCl_3$–MeOH (92:8, fractions 102–169) afforded **3** (89 mg).

NMR spectra

^{1}H and ^{13}C NMR spectra were measured on a Bruker AMX 400 (**1a**, **2** and **3**) or AC 200 (**4**) spectrometer operating at 400/200 and 100/50 MHz, respectively, using $CDCl_3$ as solvent [approximately 10–15 mg of sample were dissolved in 0.5 ml of $CDCl_3$ (**1a**, **3** and **4**) or pyridine-d_5 (**2**) and transferred into a 5 mm NMR tube], internal lock and residual $CHCl_3$ (δ_H 7.24) and $^{13}CDCl_3$ (δc 77.00) signals used as references for **1a**, **3** and **4** and residual hydrogens [δ_H 8.60 (2H-2,6), 7.00 (2H-3,5) and 7.60 (H-4)] in pyridine-d_5 [δ_c149.80 (2C-2, 6), 123.60 (2C-3,5) and 135.70 (C-4)] for **2**. One-dimensional ^{1}H and ^{13}C NMR spectra were acquired

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR for 3β,19α,23-trihydroxyurs-12-en-28-oic acid (**2**), including results obtained by heteronuclear 2D shift-correlated HMQC ($^1J_{CH}$) and HMBC ($^nJ_{CH}$, $n = 2$ and 3), in pyridine-d_5 as solvent, chemical shifts (δ, ppm) and coupling constants (J, Hz, in parentheses)[a]

	HMQC		HMBC		
Atom	δ_C[b]	δ_H	$^2J_{CH}$	$^3J_{CH}$	^{1}H × ^{1}H-NOESY
C					
4	43.3 (42.9)	—	H-3, H-5, 2H-23, 3H-24		—
8	40.9 (40.4)	—	3H-26	3H-27, H-15	—
10	37.7 (37.3)	—	H-5, H-9, 3H-25		—
13	140.4 (140.4)	—	3H-27		—
14	42.6 (42.2)	—	3H-27, H-15	H-12, H-18, 3H-26	—
17	48.8 (48.3)	—	H-18, H16		—
19	73.2 (72.7)	—	H-18, 3H-30, HO-19	3H-29	—
28	181.1 (180.7)	—		H-16, H-18	—
CH					
3	74.1 (73.7)	4.22 (dd,		3H-23, 3H-24	
5	49.2 (48.8)	1.53		H-23, 3H-25, H-3, 3H-24	2H-23α
9	48.3 (47.9)	1.95 (br t, 8.8)	2H-11	H-5, 3H-26, H-12, 3H-25)	
12	128.5 128.1)	5.64 (br s)		H-18	H-18β
18	55.1 (54.7)	3.08 (s)		H-12, H-22, 3H-30, HO-19	H-12
20	42.8 (42.4)	1.55	3H-29	3H-30	
CH_2					
1	39.8 (38.9)	1.62, 1.10	H-2	3H-25	
2	28.2 (27.7)	2.05–1.85, 1.45	H-1		
6	19.2 (18.9)	1.70 (H-6β)	H-5		3H-25
		1.45 (H-6α)			
7	33.8 (33.4)	1.70, 1.45	2H-6	H-5, 3H-26	
11	24.5 (24.1)	2.05 (H-11α, H-11β)	H-12, H-9		
15	29.8 (29.4)	2.35 (dt, 13.6, 4.1, H-15β)	H-16	3H-27	3H-26
		1.30 (H-15α)			
16	26.9 (26.5)	3.12 (dt, 13.6, 4.9, H-16α)	H-15	H-18	3H-27
		2.03 (H-16β)			
21	27.4 (27.0)	2.05, 1.40		3H-29	
22	38.8 (38.5)	2.16 (H-22α)			
		1.40 (H-22β)			
23	68.6 (68.2)	4.19 (d, 10.3, H-23a)		H-3, 3H-24	H-5α, 3H-24
		3.74 (d, 10.3, H-23b)			H-5α, 3H-24
CH_3					
24	13.5 (13.1)	1.08 (s)		H-3, H-5, 2H-23	
25	16.5 (17.3[c])	1.02 (s)		H-1, H-5, H-9	
26	17.8 (16.8[c])	1.16 (s)		H-9	
27	25.2 (24.9)	1.71 (s)		H-7, H-15	
29	27.6 (27.2)	1.14 (d, 6.6)		H-21	
30	17.2 (16.0[c])	1.46 (s)			
HO-19	—	5.00 (s)			

[a] Superimposed ^{1}H signals are described without multiplicity.
[b] Values (in parentheses) described in the literature[2] for the methyl ester derivative (**2a**).
[c] Chemical shifts marked with the same letter can be interchanged.

under standard conditions. Standard pulse sequences were used for 2D ^{1}H × ^{1}H-COSY (PO = 45 or 90°). For ^{1}H × ^{1}H-NOESY the mixing time varied between 0.5 and 1.2 s. Two-dimensional inverse hydrogen detected heteronuclear shift correlation spectra were obtained by the HMQC pulse sequence ($J_{CH} = 150$ MHz). Two-dimensional inverse hydrogen detected heteronuclear long-range correlation experiments were carried out with HMBC pulse sequence (MJ = 70 ms for $J_{CH} = 7$ Hz). Data processing was carried out on an Aspect X32 computer with UXNMR software using Bruker (AMX 400) microprograms.

RESULTS AND DISCUSSION

Comparative analysis of the PND-^{13}C NMR and DEPT-^{13}C NMR spectra[7] of each of the four triterpenoids (**1a**–**4**) was used to identify the signals corre-

Table 3. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR for 3-oxoolean-18-en-28-oic acid (**3**), including results obtained by heteronuclear 2D shift-correlated HMQC ($^1J_{CH}$) and HMBC ($^nJ_{CH}$, $n = 2$ and 3), in $CDCl_3$ as solvent and residual $CHCl_3$ used as internal reference (δ_H 7.24 and δ_C 77.00), chemical shifts (δ, ppm) and coupling constants (J, Hz, in parentheses)[a]

	HMQC		HMBC		
Atom	δ_C[b]	δ_H	$^2J_{CH}$	$^3J_{CH}$	$^1H \times {}^1H$-NOESY
C					
3	218.5 (−)	—	2H-2	H-1, 3H-23, 3H-24	—
4	47.5 (38.8)	—	H-5, 3H-24		—
8	40.7 (40.6)	—	3H-26	3H-27	—
10	37.1 (37.1)	—	H-5, H-9, 3H-25	2H-2	—
14	42.8 (42.5)	—	3H-27	3H-26	—
17	48.1 (48.1)	—			—
18	136.7 (136.9)	—	H-19		—
20	32.2 (32.0)	—	H-19, 3H-30		—
28	182.8 (176.8)	—			—
CH					
3	— (78.3)				
5	55.1 (55.4)	1.37		2H-1, 3H-23, 3H-24, 3H-25	
9	50.6 (51.1)	1.38		3H-25, 3H-26	3H-27
13	41.6 (41.2)	2.25 (br d, 11.6)		H-19, 3H-27	H-18β, H-15β, 3H-26
19	133.4 (132.3)	5.16 (s)			3H-27, 3H-29, 3H-30
CH_2					
1	40.0 (38.8)	1.94 (H-1α)	2H-2	3H-25	3H-25
		1.43 (H-1β)			
2	34.2 (27.3)	2.6–2.4	H-1		
6	19.8 (18.2)	1.65 (H-6α)	H-5		
		1.45 (H-6β)			3H-24, 3H-25, 3H-26
7	33.6 (34.5)	1.50 (H-7β)	2H-6	3H-26	
		1.42 (H-7α)			3H-27
11	21.7 (20.9)	1.55 (H-11α)			
		1.05 (H-11β)			H-13β, 3H-26
12	26.2 (25.9)	1.63 (H-12α)			H-19
		1.27 (H-12β)			
15	29.5 (29.3)	1.65 (H-15β)		3H-27	H-13β, 3H-26
		1.23 (H-15α)			
16	33.5 (33.5)	2.01 (H-16β)			
		1.40 (H-16α)			3H-27
21	33.3 (33.5)	2.17 (br d, 13.4, H-21α)		H-19, 3H-29, 3H-30	3H-30
		1.65 (H-21β)			
22	33.8 (33.5)	2.01, 1.60			
CH_3					
23	27.0 (27.9)	1.08 (s)		H-5, 3H-24	
24	21.1 (16.6)	1.02 (s)		3H-23	
25	16.7 (15.4[c])	0.95 (s)		2H-1	H-1β, H-2β, H-11β
26	16.0 (15.9[c])	1.01 (s)		H-9	H-11β, H-13β, H-15β
27	15.0 (14.9)	0.79 (s)		H-15	H-9α, H-16α, H-19
29	30.5 (30.3)	1.00 (s)		H-19, 3H-30	H-19
30	29.8 (29.4)	0.97 (s)		3H-29	H-19

[a] Superimposed ^{1}H signals are described without multiplicity.
[b] Values (in parentheses) described in the literature[2] for methylmorolate (**3a**).
[c] Chemical shifts marked with the same letter can be interchanged.

sponding to quaternary, methine, methylene and methyl carbon atoms.

^{1}H and ^{13}C NMR resonance assignments of the natural triterpenes (**2**–**4**) and the acetyl derivative of **1** (**1a**) were also carried out by 2D shift-correlated NMR techniques, $^1H \times {}^1H$-COSY, $^1H \times {}^1H$-NOESY (**2** and **3**), HMQC [$^1H \times {}^{13}C$-COSY-$^1J_{CH}$ (^{1}H detected, reverse method), **2** and **3**], $^{13}C \times {}^1H$-COSY–$^1J_{CH}$ [(^{13}C detected, conventional method), **4**], HMBC [$^1H \times {}^{13}C$-COSY–$^nJ_{CH}$, $n = 2$ and 3 (^{1}H detected, reverse method), **2** and **3**] and $^{13}C \times {}^1H$-COSY–$^nJ_{CH}$ [$n = 2$ and 3, COLOC (^{13}C detected, conventional method), **4**].[7,8] The analysis of these spectra was facilitated by comparison with ^{13}C NMR literature data (in parentheses) for the corresponding triterpenoid (**1**, Table 1, and **4**, Table 4) or methyl ester derivative of **2** (**2a**, Table 2) or

methylmorolate (**3a**, Table 3). The data on the chemical shifts of hydrogen and carbon atoms, including 2D heteronuclear [$^1J_{CH}$; $^2J_{CH}$ and $^3J_{CH}$ (Tables 2–4)] and 2D homonuclear ^{1}H × ^{1}H-NOESY (Tables 2 and 3) correlations, are summarized in Tables 1–4. The results of this study were also used to confirm and eliminate some wrong assignments reported in the literature (Tables 2–4).

The ^{1}H × ^{1}H-NOESY spectrum of **1a** provided information about the spatial proximity (dipolar coupling) between the hydrogen atoms (Table 1), allowing us to define the configuration shown in the conformers **1a** and **1b**. The *E* configuration indicated for the double bond localized between the carbon atoms CH-17 and C-20 was confirmed by the NOE observed between H-13 (δ_H 2.78) and 3H-21 (δ_H 1.79) together with that of H-17 (δ_H 5.19) and H-23 (δ_H 6.34), whereas the *E* configuration at C-22 and C-23, established by the coupling constant [J = 15.3 Hz, *trans* relationship between H-22 (δ_H 6.07) and H-23 (δ_H 6.34)], revealed, as expected, an NOE between H-22 (δ_H 6.07) and H-24 (δ_H 5.82). The difference between conformations **1a** (cisoid) and **1b** (transoid) involving the σ-bond at C-20 and CH-22 and the two double bonds at 17 (20) and 22 of the conjugated triene system of the side chain sustained by carbon C-17 is as follows: cisoid (**1a**) was deduced from

Table 4. ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR for 7-oxofriedelin (**4**), including results obtained by heteronuclear 2D shift-correlated ^{13}C × ^{1}H-COSY–$^1J_{CH}$ and ^{13}C × ^{1}H-COSY–$^nJ_{CH}$ (n = 2 and 3), in $CDCl_3$ as solvent and residual $CHCl_3$ used as internal reference (δ_H 7.24 and δ_C 77.00), chemical shifts (δ, ppm) and coupling constants (J, Hz, in parentheses)[a]

	^{13}C × ^{1}H-COSY–$^1J_{CH}$		^{13}C × ^{1}H-COSY–$^nJ_{CH}$	
Atom	δ_C [b]	δ_H	$^2J_{CH}$	$^3J_{CH}$
C				
3	211.1 (210.6)	—	3H-23	
5	47.1 (47.0)	—	3H-24	3H-23
7	210.5 (210.7)	—		2H-6, H-8
9	42.5 (42.4)	—	3H-25	
13	41.0 (39.4)	—	3H-27	3H-26
14	37.5 (37.5)	—	3H-26	3H-27
17	34.7 (30.1)	—	3H-28	
20	28.2 (28.0)	—	3H-29, 3H-30	
CH				
4	57.9 (57.8)	2.50	3H-23	3H-24
8	63.6 (63.4)	2.83 (br s)		3H-25, 3H-26
10	59.0 (59.0)	2.05		3H-24, 3H-25
18	41.8 (41.8)	1.55		3H-27, 3H-28
CH_2				
1	21.7 (21.6)	2.10		
2	40.9 (40.8)	2.40		
6	56.9 (56.9)	2.21, 2.32		3H-24
11	36.4 (35.5)	1.50		3H-25
12	31.9 (29.8[c])	1.30		3H-27
15	30.2 (31.6[c])			3H-26
16	35.5 (36.3)			3H-28
19	35.0 (34.9)			3H-29, 3H-30
21	32.9 (32.8)			3H-29, 3H-30
22	39.5 (38.6)			3H-28
CH_3				
23	6.9 (6.8)	0.85 (d, 6.5)		
24	15.3 (15.1)	0.74 (s)		
25	19.6 (18.2[d])	0.87 (s)		
26	19.8 (19.2[d])	1.38 (s)		
27	18.3 (19.4[d])	1.02 (s)		
28	31.7 (32.1)	1.10 (s)		
29	32.2 (31.8)	0.95 (s)		3H-30
30	34.7 (34.6)	0.90 (s)		3H-29

[a] Superimposed ^{1}H signals are described without multiplicity.
[b] Values (in parentheses) described in the literature[2] for 7-oxofriedelin (**4**).
[c,d] Chemical shifts marked with same letter can be interchanged.

the NOE shown by correlation of cross peaks corresponding to signals at δ_H 1.79 (3H-21) and δ_H6.07 (H-22) together with δ_H 6.34 (H-23) and δ_H 5.19 (H-17); transoid (**1b**) is consistent with an NOE between 3H-21 (δ_H 1.79) and H-23 (δ_H 6.34). The stereochemistry of the δ-lactone (**1c**: CH_2-18α and CH_2-15β) moiety was also established by an NOE observed between H-18β (*exo*, δ_H 4.37) and both H-9 (δ_H 0.78) and H-7 (δ_H 1.35) as well as H-15β (δ_H 2.44) and H-13β (δ_H 2.78) and 3H-30 (δ_H 1.03). Additional dipolar interactions are summarized in Table 1.

The stereochemistry of the chiral carbon C-13 of **3**, sustaining hydrogen atom H-13 (δ_H 2.25) in an axial orientation (H-13β), was defined on the basis of the coupling constant value ($J = 11.6$ Hz, axial–axial interaction) observed in the ^{1}H NMR spectrum and the NOE with 3H-26 (δ_H 1.01) revealed by $^1H \times {}^1H$-NOESY spectrum (Table 3).

Finally, our attention was directed towards the deshielding revealed by the singlet signal of 3H-27 (δ_H 1.71) observed in the ^{1}H NMR spectrum of **2** in pyridine-d_5 as solvent. This significant deshielding may be attributed to an anisotropic effect produced by the heteroaromatic pyridine ring involved in a hydrogen bond with the hydroxy group (δ_H 5.00, s) sustained by carbon atom C-19 as shown in **2b**. In fact, the HMBC spectrum of **2** clearly showed connectivities of C-18 (δ_C 55.1, $^3J_{CH}$) and C-19 (δ_C 73.2, $^2J_{CH}$) with the HO-19 hydrogen (δ_H 5.00). These heteronuclear spin–spin interactions via two ($^2J_{CH}$) and three ($^3J_{CH}$) bonds were used to confirm this hypothesis, in accordance with the absence of chemical exchange for the HO-19 hydrogen. The HO-3 hydrogen does not show analogous hydrogen bonding with the pyridine ring.

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