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^{1}H AND ^{13}C NMR SPECTRA OF PYROCINCHOLIC ACID

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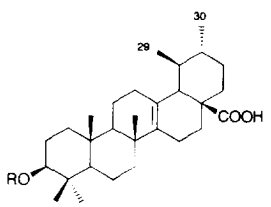
Key Word Index—*Isertia haenkeana*; Rubiaceae; leaves; 27-nor-olean-13-en-3β-acetyloxy-28-oic acid; 27-nor-olean-13-en-3β-hydroxy-28-oic acid; ^{1}H and ^{13}C NMR spectroscopy.

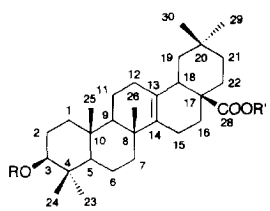
Abstract—The first NMR study of a 27-nor-triterpene of the β-amyrin type is described. In addition to conventional 1D NMR methods, 2D shift-correlated NMR techniques (DQF COSY, TOCSY, HMQC and HMBC) were used for the complete ^{1}H and ^{13}C NMR resonance assignments of 27-nor-olean-13-en-3-acetyloxy-28-oic acid and pyrocincholic acid.

INTRODUCTION

The ^{13}C NMR spectra of triterpene acids of the urs-12-ene and olean-12-ene types have been well documented and the stereochemical dependence of ^{13}C chemical shifts in the two series reported [1–5]. Recently, we have reported the complete ^{1}H and ^{13}C resonance assignment of pyroquinovic acid (**1**, **1a**) [6] and we report now a full assignment of the ^{1}H and ^{13}C NMR signals of pyrocincholic acid (**2**) and its acetate (**2a**) and ethyl ester (**2b**).



R
1 H
1a COMe



	R	R'
2	H	H
2a	$^{31}_{32}$COMe	H
2b	H	$^{31}_{32}CH_2Me$

RESULTS AND DISCUSSION

The complete assignments of the ^{1}H and ^{13}C NMR signals were carried out by 2D shift-correlated NMR techniques. All protons could be identified by using the DQF COSY spectrum, which is used for determining the connectivities between protons on the basis of geminal and vicinal couplings [7], and TOCSY experiment, which can provide evidence for two-, three-, four- and even five-bond H–H correlations [8]. The determination of the cheminal shifts of the protons and protonated carbons was made on the basis of the study of the HMQC spectrum [9]. The assignment of the non-protonated carbon signals is accomplished by using the HMBC spectrum [10].

27-*Nor-olean*-13-*en*-3β-*acetyloxy*-28-*oic-acid* (**2a**)

The ^{1}H NMR spectrum showed a signal at δ4.39 (*dd*, $J_{H\text{-}3,H\text{-}2\beta}$ = 11.5 Hz and $J_{H\text{-}3,H\text{-}2\alpha}$ = 5.0 Hz) corresponding to proton H-3α and a dense region (δ0.6–2.4) where it was impossible to resolve the individual lines even with a 500 MHz spectrometer. The ^{13}C NMR spectrum contained 31 individual signals corresponding to seven CH_3, 11 CH_2, 4 CH groups and nine quaternary carbon atoms, that were confirmed by means of a DEPT experiment. Using the chemical shift values it was fairly easy to establish the signals corresponding to CH-3 (δ_H4.39 and δ_C81.03), two CO groups (δ_C183.85 and 171.01) and the olefinic fragment C-13–C-14 (δ_C136.48 and 130.19). The assignments of the remaining signals were carried out by 2D NMR techniques.

Ring A. A DQF COSY spectrum showed connectivities between proton H-3 and the two protons H-2 (δ1.59 and 1.54). The TOCSY experiment was used to establish the signals corresponding to the protons H-1 (δ1.66 and 0.93). The assignments of the carbon signals were carried out with a HMQC spectrum: δ37.77 (C-1) and 23.63 (C-2). The HMBC spectrum showed linkages between the proton H-3 and the carbon atoms C-31 (δ171.01), C-1, C-4 (δ37.74), C-5 (δ55.28) and the methyl groups Me-23 (δ27.96) and Me-24 (δ16.41). The difference between the two values for the methyl groups Me-23 and Me-24 has been rationalized by comparison with the ^{13}C NMR spectral data for known oleanane derivatives [2, 11]; in

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addition, the shifts of such methyls are also diagnostically sensitive to the relative stereochemistry of adjacent substituents. For example, the shift of an equatorial methyl gauche to γ-OH (*ca* 27 ppm) is quite different when compared with an axial methyl gauche to an equatorial γ-OH (*ca* 18 ppm) [12].

Ring B. Beginning from the signal corresponding to the proton H-5 and with the DQF COSY, TOCSY and HMQC spectra were carried out the assignments of CH_2-6 (δ_H1.48 and 1.31, δ_C18.44) and CH_2-7 (δ_H1.71 and 1.09, δ_C39.03). The HMBC spectrum correlated the protons H-7 with the proton H-9 (δ_H0.93, δ_C55.98).

Rings C and D. The assignments of ring C were achieved from H-9 by using DQF COSY, TOCSY and HMQC experiments. In this way signals were assigned corresponding to CH_2-11 (δ_H1.49 and 1.36, δ_C17.68) and CH_2-12 (δ_H2.11 and 1.76, δ_C31.48). The HMBC spectrum showed connectivities between the two protons H-11and C-13 (δ130.19), and also between the C-13 and the two protons H-15 (δ_H2.06 and 1.92, δ_C20.42). The assignment of CH_2-16 (δ_H1.84 and 1.60, δ_C22.67) was made from the signal corresponding to H-15 by using the COSY and HMQC spectra.

Ring E. The signals corresponding to ring E were assigned by using the HMBC spectrum. In this spectrum the carboxylic acid (C-28) showed correlation with the proton H-18 (δ_H2.29, *dd*, J = 12.7 and 3.9 Hz; δ_C38.92). In addition, the proton H-18 showed connectivities with C-12, C-13, C-14 (δ136.48), C-16, C-19 (δ41.14), C-20 (δ30.51) and C-22 (δ31.10). The assignment of the remaining signals of ring E were carried out with the DQF COSY and HMQC spectra. The methyl groups Me-29 (δ32.54) and Me-30 (δ24.66) were assigned by comparison with the ^{13}C NMR spectra data for known oleanane derivatives [2, 11]. The results obtained are summarized in Table 1.

Pyrocincholic acid (**2**) *and its ethyl ester* (**2b**)

The assignment of the ^{13}C signals corresponding to compounds **2** and **2b** were made by comparison with the ^{13}C NMR spectrum of acetylated derivative **2a**. The different substitution at C-3, showed chemical shifts which are in accordance with the values reported in the literature for the ring A oleanane skeleton-based compounds; for example, oleanolic acid (olean-12-en-3β-hydroxy-28-oic acid) [13]: δ(ppm) 27.2 (C-2) and 79.4 (C-3).

Carbon-13 NMR spectroscopy can be used and it is the most precise tool for distinguishing the pentacyclic triterpenes of the α-amyrin and β-amyrin types, which is

Table 1. NMR data for 27-nor-olean-13-en-3β-acetyloxy-28-oic acid (**2a**)

No.	δ ^{13}C (ppm)	HMQC (^{1}H, ppm)	COSY	TOCSY	**2** (**2b**) (^{13}C, ppm)
1	37.77	1.66, 0.93	H2, H2′	H2, H2′, H3	38.08 (38.08)
2	23.63	1.59, 1.54	H1, H1′, H3	H1, H1′, H3	27.23 (27.27)
3	81.03	4.39	H2, H2′	H2, H2′, H1, H1′	79.06 (79.03)
4	37.74				38.97 (38.80)
5	55.28	0.78	H6, H6′	H6, H6′, H7′ H7′	55.14 (55.12)
6	18.44	1.48, 1.31	H5, H7, H7′	H5, H7, H7′	18.53 (18.51)
7	39.03	1.71, 1.09	H6, H6′	H6, H6′, H5	39.16 (39.19)
8	37.19				37.18 (37.22)
9	55.98	0.93	H11, H11′	H11, H11′, H12, H12′	56.11 (56.16)
10	37.54				37.51 (37.46)
11	17.68	1.49, 1.36	H9, H12, H12′	H9, H12, H12′	17.24 (17.63)
12	31.48	2.11, 1.76	H11, H11′	H11, H11′, H9	31.60 (31.63)
13	130.19				130.28 (130.37)
14	136.48				136.53 (136.37)
15	20.42	2.06, 1.92	H16, H16′	H16, H16′	20.37 (20.39)
16	22.67	1.84, 1.60	H15, H15′	H15, H15′	22.91 (22.86)
17	44.99				44.96 (44.97)
18	38.92	2.29	H19, H19′	H19, H19′	38.74 (38.96)
19	41.14	1.40, 0.95	H18	H18	41.36 (41.39)
20	30.51				30.51 (30.52)
21	33.99	1.29, 1.14	H22, H22′	H22, H22	34.02 (34.00)
22	31.10	1.77, 1.44	H21, H21′	H21, H21′	31.15 (31.19)
23	27.96	0.77			27.91 (27.93)
24	16.41	0.78			16.30 (16.35)
25	16.39	0.78			15.25 (15.25)
26	20.35	0.80			20.42 (20.40)
28	183.85				183.34 (177.83)
29	32.54	0.83			32.68 (32.67)
30	24.66	0.85			24.62 (24.67)
31	171.01				(59.91)
32	21.27	1.96			(14.32)

not easy otherwise. The differences between both series have been well documented in the literature [1, 2]. We report herein a comparative ^{13}C NMR study between **1** and **2**. These compounds belong to α- and β-amyrin types, respectively, but present a double bond rearrangement from Δ^{12} to Δ^{13}.

The major differences between both compounds were found in the chemical shifts for the C-12 and for the carbon atoms belonging to ring E. In general, C-12 is deshielded (*ca* 2–3 ppm) in the α-amyrin type compared to the corresponding β-amyrin type. C-12 in **2** resonated at about 3.6 ppm upfield compared to that in **1**, indicating that the 19β (equatorial)-methyl group was involved in strong steric interaction with C-12. The δ-deshielding effect noted here can be compared with the values reported in the literature [1, 2, 14–16].

The ring E carbon atom which shows the highest $\Delta\delta$ value between both series is C-18, whose absorption is upfield shifted ($\Delta\delta$ 11.2) in **2** due to a shielding effect from the 20β(axial)-methyl group which is γ-gauche disposed to C-18. The C-29 of **1** showed two shielding γ-gauche interactions with C-13 and C-30, as a result of which the chemical shift C-29 and C-30 are observed at low field compared to those in **2**; C-29 (*ca* $\Delta\delta$12.7) and C-30 (*ca* $\Delta\delta$6.9). The differences in ^{13}C shift for the other carbon atoms of the ring E are in accordance with the values reported in the literature for both series [1–5].

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were carried out on a 500 spectrometer operating at 500 and 125 MHz, respectively, using $CDCl_3$ as solvent at 30°. One-dimensional ^{1}H and ^{13}C spectra were acquired under standard conditions. DQF COSY 2D NMR spectra were acquired in the phase-sensitive mode. Data were collected in a 1024×256 matrix with a spectral width of 2485 Hz and 2 sec of relaxation delay and processed in a 1024×1024 matrix. The TOCSY experiments were acquired with mixing times of 20–80 msec and processed in the phase-sensitive mode using parameters very similar to those given above for the DQF COSY experiments. 2D inverse proton detected heteronuclear shift correlation spectra were obtained using the HMQC pulse sequence. Data were collected in a 1024×256 matrix with a spectral width of 2485 Hz in the proton domain and 10000 Hz in the carbon domain and processed in a 1024×512 matrix. The null time following the BIRD pulse was 400 msec. 2D inverse proton detected heteronuclear long-range shift correlation experiments were carried out with the HMBC pulse sequence. The experiments were optimized for long-range coupling constants of 8 Hz and the data processed using parameters very similar to those used in the HMQC experiments.

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