MAGNETIC RESONANCE IN CHEMISTRY VOL. 35, 802–805 (1997)

NOTE

Total Assignment of ^{1}H and ^{13}C NMR Spectra of Two Isomeric Triterpenoids

F. J. Q. Monte,[1]* J. P. Kintzinger[2] and R. Braz-Filho[3]

[1] Departamento de Química Orgânica e Inorgânica, Centro de Ciências, Universidade Federal do Ceará, 60021-970-Fortaleza, Brazil

[2] Laboratoire de RMN et de Modelisation Moléculaire, UA 422 du CNRS, Faculté de Chimie, Université Louis Pasteur, 67008 Strasbourg, France

[3] Setor de Química de Produtos Naturais, Laboratório de Tecnologia de Alimento, Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense, 28015-620-Campos-RJ, Brazil

The complete ^{1}H and ^{13}C assignment of two closely related isomeric triterpenoid derivatives (methyl 2α,3β-di-*O*-acetylolean-12-en-28-oate and methyl 2α,3α-di-*O*-acetylurs-12-en-28-oate) is described. In addition to 1D NMR methods, 2D shift-correlated NMR techniques (COSY, NOESY, HMBC and HMQC) were used for the assignment. © **1997 John Wiley & Sons, Ltd.**

Magn. Reson. Chem. **35**, 802–805 (1997) No. of Figures: 0 No. of Tables: 2 No. of References: 10

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

Received 22 April 1997; revised 17 June 1997; accepted 17 June 1997

INTRODUCTION

Triterpenoids are secondary metabolites detected in terrestrial and marine flora and fauna, with great potential as drugs.[1] They are also utilized as biological markers of lipidic nature in organic geochemistry.[2,3]

The complete ^{1}H and ^{13}C NMR spectral assignment of this important class of natural products serves to build up a database for the elucidation of novel triterpenoids and is also useful in fully understanding the correlations between their molecular conformation and biological activity.

In the series of pentacyclic triterpenoids, earlier reports on complete ^{1}H and ^{13}C spectral assignment include the use of indirect heteronuclear shift-correlated pulse sequence (XCORFE),[4] 2D $^{1}H \times ^{13}C$ shift correlated spectroscopy ($^{13}C \times ^{1}H$–HETCOR) in combination with homonuclear correlation spectroscopy ($^{1}H \times ^{1}H$–COSY) and two-dimensional nuclear Overhauser effect (NOESY)[5] and, more recently, total correlation spectroscopy (TOCSY) in association with $^{1}H \times ^{1}H$–COSY, heteronuclear multiple-bond coherence ($^{1}H \times ^{13}C$–COSY–^{2}J and $^{3}J_{CH}$–HMBC) and heteronuclear multiple-quantum coherence ($^{1}H \times ^{13}C$–COSY–$^{1}J_{CH}$–HMQC).[6]

In this paper we report an extensive NMR study of two isomeric triterpenoid derivatives ($C_{35}H_{54}O_6$) where complete ^{1}H and ^{13}C resonance assignments by application of 1D and 2D spectral techniques were made. The following steps were employed: (a) application of the HMBC experiment to chemical shift assignment of the ^{13}C spectra, (b) use of the HMQC spectra to determine the chemical shifts of the hydrogen atoms and to confirm those of the hydrogenated carbons, (c) use of hydrogen $^{1}H \times ^{1}H$–COSY and $^{1}H \times ^{1}H$–NOESY maps to confirm the ^{1}H assignments (and, indirectly, also the ^{13}C assignments) and to establish the configurational assignment (α and β) of all methylene and methine hydrogens and (d) analysis of the splitting patterns (multiplicity and coupling constant) in the 1D NMR spectra to confirm the resonances (including the configurational assignment) of various hydrogen atoms. The compounds investigated were methyl 2α,3β-di-*O*-acetylolean-12-en-28-oate (**1**) and methyl 2α,3α-di-*O*-acetylurs-12-en-28-oate (**2**), obtained after acetylation and methylation of a mixture isolated from *Mentha villosa* Huds.[7] The aerial parts of this plant are used as a remedy in the treatment of amebiasis, giardiasis[8] and shistosomiasis.[9]

EXPERIMENTAL

Plant material

Mentha villosa was collected in the Horto de Plantas Medicinais da Universidade Federal do Ceará, Fortaleza, Brazil. A voucher of the plant (No. 16.545) is deposited in the Herbarium Prisco Bezerra do Departmento de Biologia da Universidade Federal do Ceará.

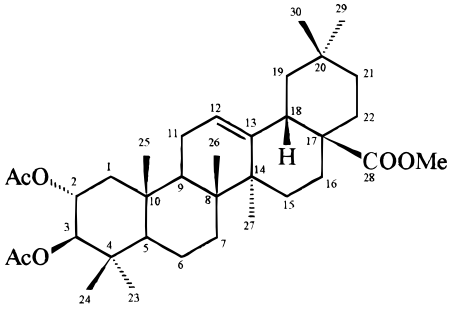
NMR spectra

^{1}H and ^{13}C NMR experiments were performed on a Bruker ARX 500 spectrometer operating at 500.1 MHz for hydrogen and 125.75 MHz for ^{13}C, using $CDCl_3$ as solvent. Solutions were made from 0.35 ml of $CDCl_3$ and 2–8 mg of triterpenes with TMS as the internal

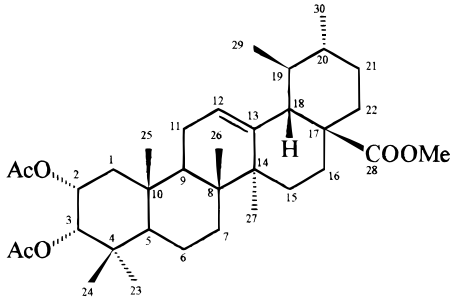
* *Correspondence to*: F. J. Q. Monte.

Contract grant sponsor: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

CCC 0749–1581/97/110802–04 $17.50



1



2

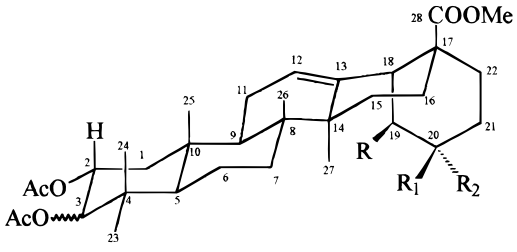
standard. For all experiments the temperature was stabilized at 298 K. For the NOESY experiments samples were degassed by bubbling nitrogen through the solution and fitting a PTFE serum cap. The 2D experiments were acquired and processed with the software provided by Bruker on an Aspect X32 instrument.

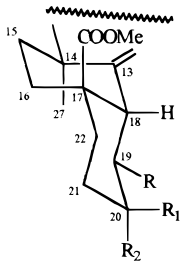
Typical acquisition and processing conditions for COSY and NOESY experiments were a relaxation

Table 1. NMR data for methyl 2α,3β-di-*O*-acetylolean-12-en-28-oate (1)[a]

		^{1}H × ^{13}C–HMQC ($^1J_{CH}$)	^{1}H × ^{13}C–HMBC		^{1}H × ^{1}H–NOESY
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	
4	39.37	—	3H-23, 3H-24		—
8	39.35	—	H-7β, H-9, 3H-26	2H-11, 3H-27	—
10	38.17	—	H-1β, 3H-25	2H-11	—
13	143.90	—	H-12, H-18	2H-11, H-15α, H-19α, 3H-27	—
14	41.69	—	H-15β, 3H-27	H-12, H-18, 3H-26	—
17	46.71	—	H-18, 2H-22		—
20	30.73	—	3H-29, 3H-30	2H-22	—
28	178.20	—		H-16α, H-18, H-22β, MeO-28	—
AcO-2	170.30	—	H_3CCO_2-2	H-2	—
AcO-3	171.0	—	H_3CCO_2-3	H-3	—
CH					
2	70.05	5.09 (ddd, J = 10.5, 10.3, 4.5 Hz, H-2β)	2H-1, H-3		H-1β, 3H-24, 3H-25
3	80.66	4.73 (d, J = 10.3 Hz, H-3α)	H-2	2H-1, 3H-23, 3H-24	H-5α
5	54.92	0.95 (d, J = 10.3 Hz, H-5α)	H-6β	H-1α, H-3, H-7β, 3H-25	H-3α, H-9
9	47.60	1.60 (H-9α)	2H-11	H-7β, H-12, 3H-25, 3H-26	H-1α, H-3α, H-5α, 3H-27
12	121.97	5.26 (t, J = 3.5 Hz)	2H-11	H-18	2H-11, H-18
18	41.27	2.86 (dd, J = 15.6, 5.2 Hz, H-18β5)	2H-19	H-12, 2H-16, 2H-22	H-12, H-19β, H-22β, 3H-30
CH_2					
1	43.91	2.00 (dd, J = 12.3, 4.5 Hz, H-1β)	H-2	H-3, 3H-25	H-1α, H-2β, H-12
		1.03 (H-1α)			H-1β, H-3α, H-9α
6	18.25	1.55 (H-6α)	2H-7		6β, 7α, 3H-23
		1.40 (H-6β)			6α, 3H-24, 3H-25, 3H-26
7	32.38	1.44 (dt, J = 11.4, 3.1 Hz, H-7α)		3H-26	6α, 7β, 3H-27
		1.28 (H-7β)			7α, 3H-26
11	23.50	1.90–1.85 (H-11α, H-11β)	H-9, H-12		H-12
15	27.65	1.59 (H-15β)	H-16α	3H-27	3H-26
		1.05 (H-15α)			H-16α
16	23.04	1.96 (H-16α)		H-18, 2H-22	21α, H-15α, H-16β, 3H-27
		1.60 (H-16β)			H-16α
19	45.85	1.61 (H-19α)		3H-29, 3H-30	H-21α
		1.14 (H-19β)			H-18
21	33.80	1.33 (dt, J = 12.4, 4.2 Hz, H-21α)	2H-22	3H-29, 3H-30	H-19α
		1.18 (H-21β)			3H-29
22	32.40	1.70 (dt, J = 12.9, 4.6 Hz, H-22β			H-18, H-22α, 3H-30
		1.50 (H-22α)			H-22β
CH_3					
23	28.45	0.90 (s)		H-3, 3H-24	H-6α
24	17.65	0.88 (s)		H-3, 3H-23	H-2β, H-6β
25	16.44	1.04 (s)		2H-1, H-9	H-1β, H-6β
26	16.82	0.72 (s)		H-7β, H-9	H-6β, H-7β, H-15β
27	25.94	1.11 (s)		2H-30	H-7α, H-9, H-16α
29	33.14	0.89 (s)		3H-30	H-21β, H-22β
30	23.66	0.92 (s)		3H-29	
AcO-2	21.19	1.97 (s)			
AcO-3	20.95	2.05 (s)			
MeO-28	51.58	3.60 (s)			

[a] Multiplicity of signals of carbon atoms deduced by comparative analysis of PND and DEPT ^{13}C NMR. Chemical shifts of hydrogen atoms obtained from 1D ^{1}H NMR. The homonuclear 2D ^{1}H × ^{1}H–COSY data were also used in these assignments. J in Hz.





1a 3β-OAc, R=H, R_1=R_2=Me
2a 3α-OAc, R=Me, R_1=H, R_2=Me

3

delay of 1–2 s, 512–1024 t_1 increments, 1024–2048 t_2 points and a sweep width of 6 ppm. Sine-bell squared and shifted ($\pi/4$, $\pi/6$ and $\pi/8$) apodization functions were used for processing. The mixing time in the NOESY experiments, generally set at 1.2–1.5 s, was also varied between 0.8 and 2 s without a substantial change in the results. For $^1H \times ^{13}C$ (^{13}C detected) and $^{13}C \times ^1H$ (^1H detected) correlations, the same relaxation delay was used, 512–1024 t_1 increments, 1024–2048 t_2 points and a sweep width of 7 ppm for ^1H and

Table 2. NMR data for methyl 2α,3α-di-*O*-acetylurs-12-en-28-oate (2)[a]

		$^1H \times ^{13}C$–HMQC ($^1J_{CH}$)	$^1H \times ^{13}C$–HMBC ($^nJ_{CH}$, n = 2 and 3)		
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	$^1H \times ^1H$–NOESY
4	38.14	—	3H-23, 3H-24, H-3		—
8	39.69	—	H-9, 3H-26	2H-11, H-15β, 3H-27	—
10	38.42	—	2H-1, H-5, 3H-25		—
13	138.29	—	H-18	2H-11, 3H-27	—
14	42.00	—	H-15β, 3H-27	H-9, H-18, 3H-26	—
17	48.09	—	H-16α, H-18, H-22β		—
28	178.05	—		H-16α, H-18, H-22β	—
AcO-2	170.44	—	H_3CCO_2-2	H-2	—
AcO-3	170.70	—	H_3CCO_2-3	H-3	—
CH					
2	68.28	5.24 (dd, H-β)	2H-1, H-3		H-1β, H-3β, 3H-24, 3H-25
3	77.20	4.97 (s, H-3β)	H-2	2H-1, 3H-23, 3H-24	H-2β, 3H-23, 3H-24
5	49.67	1.17 (d, J = 12.3 Hz, H-5α)	H-6β	H-7β, H-9, 3H-23, 3H-24, 3H-25	H-1α, H-7α, H-9α
9	47.42	1.67 (H-9α)	2H-11	H-1α, H-5, 3H-25	H-1α, H-5α, H-7α, 3H-27
12	125.29	5.25 (t, J = 3.5 Hz)	2H-11	H-18	2H-11, H-18
18	52.85	2.24 (d, J = 11.3 Hz, H-18β)		3H-29	H-12, H-20β, H-22β, 3H-30
19	38.90	1.35 (H-19α)	3H-29	3H-30	3H-30
20	39.10	1.32 (H-20β)	3H-30	H-18	3H-29
CH_2					
1	39.01	1.68 (H-1β)	H-2, H-3	3H-25	H-1α, H-2β, 3H-25
		1.38 (H-1α)			H-1β, H-5α, H-9α
6	17.86	1.48 (H-6α)			H-6β, H-5α, 3H-23
		1.31 (H-6β)			H-6α, 3H-24, 3H-25, 3H-26
7	32.68	1.50 (H-7α)		H-5, 3H-26	H-5α, H-7β, 3H-27
		1.32 (H-7β)			H-7α, 3H-26
11	23.33	1.94 (m, H-11α, H-11β)	H-9, H-12		H-12, 3H-25, 3H-26
15	28.01	1.77 (dt, J = 13.4, 4.1 Hz, H-15β)	H-16α	3H-27	H-15α, 3H-26
		1.10 (H-15α)			H-15β, H-16α
16	24.23	2.00 (dt, J = 13.4, 4.1 Hz, H-16α)	H-15β	H-18, H-22β	H-15α, H-16β, 3H-27
		1.65 (H-16β)			H-16α
21	30.67	1.50 (H-21β)		3H-30	H-21α
		1.28 (H-21α)			H-16α, H-21β, H-22α
22	36.64	1.68 (H-22α)			H-21α
		1.58 (dt, J = 14.4, 4.1 Hz, H-22β)			H-18, H-22α
CH_3					
23	27.80	0.87 (s)		H-3, 3H-24	H-5α, 3H-24
24	21.67	0.98 (s)		H-3, 3H-23	H-2β, H-3β, H-6β, 3H-23
25	16.33	1.04 (s)		2H-1, H-5, H-9	H-1β, H-2β, H-6β, 2H-11
26	17.02	0.74 (s)		H-7β, H-9	H-7β, 2H-11, H-15β
27	23.77	1.12 (s)			H-7α, H-9α, H-16α
29	21.23	0.85 (d, J = 7.2 Hz)		H-18	H-18, H-20
30	16.95	0.94 (d, J = 5.2 Hz)			H-19
AcO-2	21.05	2.11 (s)			
AcO-3	21.14	1.95 (s)			
MeO-28	51.53	3.60 (s)			

[a] Multiplicity of signals of carbon atoms deduced by comparative analysis of PND and DEPT ^{13}C NMR. Chemical shifts of hydrogen atoms obtained from 1D ^1H NMR. The homonuclear 2D $^1H \times ^1H$–COSY was also used in these assignments. J in Hz.

180 ppm for ^{13}C. Lorentzian and Gaussian deconvolution were generally used in the processing. The number of scans was set for an overall acquisition time of about 12 h–16 h.

RESULTS AND DISCUSSION

The signals corresponding to quaternary, methine, methylene and methyl carbon atoms were identified by comparative analysis of the ^{13}C NMR–PND and ^{13}C NMR–DEPT spectra.

The attribution of the chemical shifts of the non-protonated and several protonated carbons was made mainly on the basis of ^{1}H × ^{13}C–HMBC [heteronuclear multiple bond connectivity: coupling of hydrogen and carbon-13 via two ($^2J_{CH}$) and three ($^3J_{CH}$) bonds] spectra. Methyl groups are strategically located in these triterpenoids, providing a network of the two- and three-bond connectivities which tie the molecule together and allow the assignment of the ^{13}C signals.[4]

The starting point for the assignments for **1** was the *gem*-dimethyl groups. These groups provide examples where the cross peaks corresponding to a methyl ^{1}H signal and a ^{13}C signal from a second methyl group ($^3J_{CH}$), 3H-23 with C-24 and 3H-24 with C-23; 3H-29 with C-30 and 3H-30 with C-29) were observed, allowing identification of the signals of 3H-23, 3H-24, 3H-29 and 3H-30. The signals of 3H-23/3H-24 also show cross peaks at ^{13}C frequencies corresponding to CH-3 (δ_C 80.66), C-4 (δ_C 39.37) and CH-5 (δ_C 54.92) and 3H-29/3H-30 to CH-19 (δ_C 45.85), C-20 (δ_C 30.73) and CH_2-21 (δ_C 33.80) (Table 1). At one of these (CH-5, δ_C 54.92), there is a third signal which corresponds to a spin–spin interaction ($^3J_{CH}$) with 3H-25 (δ_H 1.04). Similarly, the methine carbon (CH-9, δ_C 47.60) showed a cross 3H-26 (δ_H 0.72). Working along the molecule in this fashion, it was possible to assign all methyl ^{1}H signals and, by ^{1}H × ^{13}C–HMQC ($^1J_{CH}$), the corresponding ^{13}C signals (Table 1). The HMBC and COSY spectra allowed assignment of other carbons while assignment of CH_2 hydrogens as α or β was provided by NOESY spectra. These spectra also indicated that **1** was in conformation **1A** and confirmed the *cis* junction of rings D and E (see **3**).

Using the same procedure, the HMBC, COSY, NOESY and HMQC spectra furnished the ^{1}H and ^{13}C chemical shifts of **2** (Table 2); and also the molecular conformation **2A** and *cis* ring junction as in **3**.

In a report on methyl 2α,3α-dihydroxyursolate,[10] the chemical shifts of the carbon atoms CH_3-29 (δ_C 17.1) and CH_3-30 (δ_C 21.2) were consistent with those of the same carbon atoms in **2**. The ^{1}H × ^{13}C–HMBC spectrum of **2** allowed the unambiguous assignment of CH_3-29 (δ_C 16.9) by connectivities of the resonance at δ_H 2.24 (H-18) to the resonance at δ_C 16.9 (CH_3-29) and of the resonance at δ_C 52.8 (CH-18) to the resonance at δ_H 0.85 (3H-29). Consequently, the signal at δ_C 21.2 was attributed to CH_3-30.

In conclusion, the complete ^{1}H and ^{13}C chemical shift assignments of the triterpenoids **1** (Table 1) and **2** (Table 2), possessing extended proton spin systems, have been achieved based on the combined use of one-dimensional (^{1}H and ^{13}C) and two-dimensional NMR techniques (HMBC, HMQC, COSY and NOESY). The two-dimensional NOESY technique proved to be particularly useful in making configurational assignments for protons.

Acknowledgements

The authors gratefully acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Brazil) for financial support and the Laboratoire de RMN et de Modelisation Molécuilare, ULP, Strasbourg, France, for facilities provided. Special appreciation is extended to R. Graff for technical assistance with the NMR measurements and Dr V. M. Lunjanck for correcting the text.

REFERENCES

1. S. B. Mahato, A. K. Nandy and G. Roy, *Phytochemistry* **31**, 2199 (1992).
2. A. S. Mackenzie, S. C. Brassel, G. Eglington and G. R. Maxwell, *Science* **217**, 491 (1982).
3. E. P. Kenneth, J. M. Moldowan, *The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments*. Prentice Hall, Englewood Cliffs, NJ (1993).
4. W. F. Reynolds, S. McLean, J. Poplawski, R. G. Enriquez, L. I. Escobar and I. Leon, *Tetrahedron* **42**, 3419 (1986).
5. D. Loganathan, G. K. Trivedi and K. V. R. Chary, *Magn. Reson. Chem*. **28**, 925 (1990).
6. M. J. Jimeno, A. Rumbero and P. Vázquez, *Magn. Reson. Chem*. **33**, 408 (1995).
7. E. F. Oliveira, Thesis, Universidade Federal do Ceará, Fortaleza, Brazil (1995).
8. F. J. A. Matos, *Farmácias Vivas*, 2nd ed. MV e Atual, Universidade Federal do Ceará, Fortaleza, Brazil (1991).
9. M. Muniz, *Ciência Hoje* **16**(96), 68 (1990).
10. S. B. Mahato and A. P. Kundu, *Phytochemistry* **37**, 1517 (1994).