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Note

Total Assignment of ^{1}H and ^{13}C Spectra of the Chlorinated Triterpenoid (Methyl 2α,3β,24-Tri-*O*-Acetylolean-12α-Chloro-28,13β-Olide) by NMR Spectroscopy

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ABSTRACT: The current substantial interest concerning the structure elucidation of new active natural products led to the complete ^{1}H and ^{13}C chemical shift assignment of a triterpenoid derivative, methyl 2α,3β,24-tri-*O*-acetylolean-12α-chloro-28,13β-olide, obtained after acetylation and methylation of a mixture isolated from *Mentha villosa*. The 2D shift-correlated NMR techniques HMBC, HMQC, COSY and NOESY were used. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: triterpenoids; ^{1}H NMR; ^{13}C NMR; 1D NMR; 2D NMR; HMBC; HMQC; COSY; NOESY

INTRODUCTION

Biologically active natural products obtained from medicinal plants are widely used in therapeutics. It has been estimated that 80% of people living in developing countries are almost completely dependent on traditional medical practices for their primary health care needs, and higher plants are known to be the main source of drug therapy in traditional medicine.[1] Consequently, owing to the current substantial interest concerning the structure elucidation of new active natural products, it is now standard practice to conduct detailed spectroscopic analysis of the isolates in order to assign unambiguously all the protons and carbons in their NMR spectra. In the case of triterpenoids, considerable recent work strongly indicates their great potential as drugs.[2] The ^{1}H and ^{13}C chemical shifts of this important class of natural products provide useful information concerning conformations and configurations of the complex organic derivatives[3] and are also useful in fully understanding the correlations between their molecular conformation and biological activity.[4]

In this paper, we report an extensive NMR study of a triterpenoid derivative in which the complete ^{1}H and ^{13}C resonance assignment was achieved by application of 1D and 2D NMR techniques. The following steps were concurrently 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 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 assignment (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 splitting patterns (multiplicity and coupling constants) in the 1D NMR spectra to confirm the resonances (including the configurational assignment) of various hydrogen atoms. The compound investigated was methyl 2α,3β,24-tri-*O*-acetylolean-12α-chloro-28-13β-olide (**1**) (Fig. 1), isolated after acetylation and methylation of a mixture obtained from *Mentha villosa* Huds.[5] The aerial parts of this plant are used as a remedy in the treatment of amebiasis, giardiasis[6] and shistosomiasis.[7]

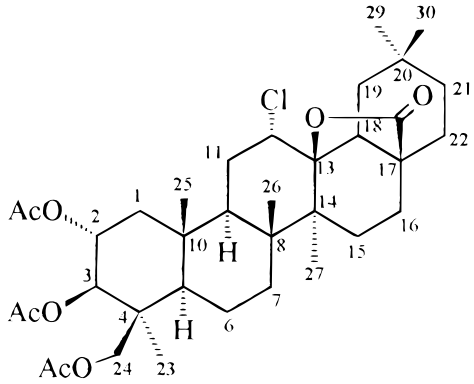

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Figure 1. Structure of 2α,3β,24-tri-*O*-acetylolean-12α-chloro-28,13β-olide (**1**).

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Table 1. NMR data for methyl 2α,3β,24-tri-*O*-acetylolean-12α-chloro-28,13β-olide (1)[a]

	$^1H \times {}^{13}C$-HMQC ($^1J_{CH}$)		$^1H \times {}^{13}C$-HMBC		
Atom	δ_C (ppm)	δ_H (ppm)	$^2J_{CH}$	$^3J_{CH}$	$^1H \times {}^1H$-NOESY
C					
4	42.70	—	H-3, 3-H23, 2H-24		—
8	42.20	—	H-9, 3H-26	3H-27	—
10	37.50	—	H-1β, H-9, 3H-25		—
13	91.40	—	H-18	3H-27	—
14	43.20	—	3H-27	H-18, 3H-26	—
17	43.00	—		H-16α	—
20	31.80	—	3H-29, 3H30		—
28	178.80	—			—
AcO-2	170.30	—	*Me*COO-2	H-2	—
AcO-3	170.60	—	*Me*COO-3	H-3	—
AcO-24	170.80	—	*Me*COO-24	2H-24	—
CH					
2	69.20	5.18 (dt, J = 10.4, 4.1 Hz, H-2β)	H-1α, H-1β, H-3		H-1β, 2H-24, 3H-25
3	79.50	4.86 (d, J = 10.4 Hz, H-3α)	H-2	H-1β, 3H-23, 2H-24	H-5α, 3H-23
5	55.20	1.15 (H-5α)		H-1β, 3H-23, 2H-24, 3H-25	H-3α, H-6α, H-7α, H-9α, 3H-23
9	45.20	1.82 (H-9α)		H-1α, H-1β, 3H-25, 3H-26	H-1α, H-5α, H-7α, 3H-27
12	63.30	4.18 (H-12β)		H-18	2H-11, H-18β
18	51.80	2.03 (H-18β)			H-12β, H-22β, 3H-30
CH_2					
1	44.00	2.17 (H-1β)		3H-25	H-1α, H-2β, H-11α, 3H-25
		1.14 (H-1α)			H-1β, H-3α, H-5α, H-9α
6	18.70	1.66 (H-6α)			H-5α, H-7α, 3H-23
		1.56 (H-6β)			H-7β, 3H-25, 3H-26
7	34.80	1.56 (H-7α)		3H-26	H-5α, H-6α, H-9α, 3H-27
		1.32 (H-7β)			H-6β, 3H-26
11	29.60	2.28 (dt, J = 13.7, 4.1 Hz, H-11β)			H-12β, H-18β, 3H-25, 3H-26
		1.72 (td, J = 13.7, H-11α)			H-1β, H-9α
15	28.80	1.93 (H-15β)		3H-27	H-11β, H-15α, 3H-26
		1.18 (H-15α)			3H-27
16	21.30	1.94 (H-16β)			H-16α
		1.30 (H-16α)			H-16β, H-19α, 3H-27
19	39.90	2.17 (H-19α)		3H-29, 3H-30	H-16α, H-21α, 3H-27, 3H-29
		2.03 (H-19β)			3H-27
21	33.90	1.31 (H-21α)		3H-29, 3H-30	H-16α, H-19α, H-22α, 3H-29
		1.27 (H-21β)			H-22β, 3H-30
22	27.60	1.67 (H-22β)			H-18β, H-21β, 3H-30
		1.61 (H-22α)			H-21α
24	65.30	4.20 (s, 2H-24)		3H-23	H-2β, H-6β, 3H-23
CH_3					
23	22.90	1.03 (s)		2H-24	H-3α, 2H-24
25	17.40	1.05 (s)			H-1β, H-2β, H-11β, 3H-26
26	18.80	1.19 (s)			H-7β, H-11β, H-15β, 3H-25
27	22.00	1.38 (s)			H-7α, H-9α, H-15α, 2H-19
29	33.40	1.00 (s)		3H-30	2H-19, H-21α, 3H-30
30	23.50	0.90 (s)		3H-29	H-18β, H-19β, H-21β, 3H-29
AcO-2	20.90	2.07 (s)			
AcO-3	20.90	2.07 (s)			
AcO-24	21.00	2.00 (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 2D $^1H \times {}^1H$-COSY and 2D $^1H \times {}^{13}C$-HMQC spectra were also used in these assignments.

This is the first report giving the complete assignment of a pentacyclic triterpenoid derivative of oleanolic type.

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 Departamento de Biologia da Universidade Federal do Ceará.

NMR Spectra

^1H 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 carbon, using $CDCl_3$ as solvent. Solutions were made from 0.35 ml of

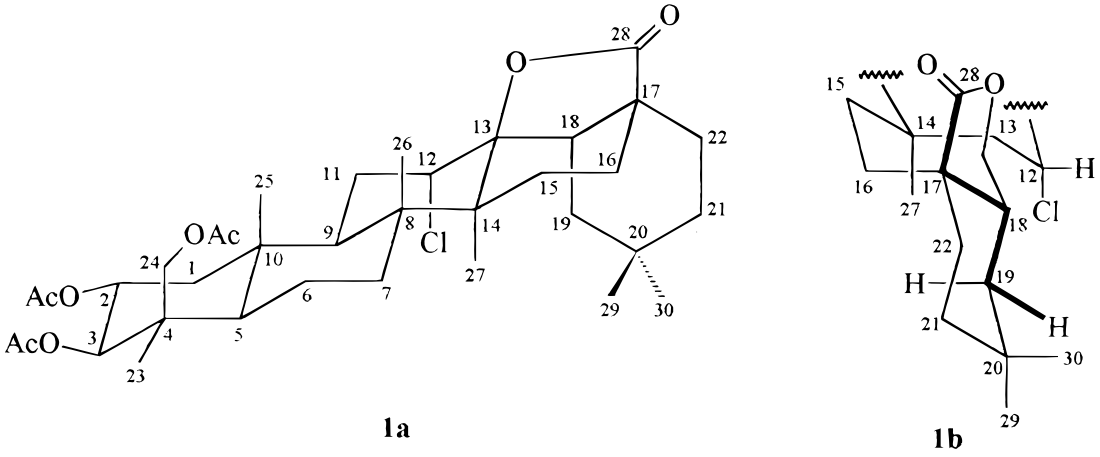

Figure 2. Stereochemical view of the structure of **1**.

$CDCl_3$ and 2–8 mg of triterpenes with TMS as the internal standard. For all experiments, the temperature was maintained 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 computer.

Typical acquisition and processing conditions for COSY and NOESY experiments were as follows: relaxation delay, 1–2 s; 512–1024 t_1 increments; 1024–2048 t_2 points; and sweep width, 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 substantial changes 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, the sweep width being 7 ppm for ^1H and 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 12–16 h.

RESULTS AND DISCUSSION

We have used inverse detection techniques to provide complete and unambiguous ^{13}C assignments.[8] Thus, the identification of the non-hydrogenated and of several hydrogenated carbon signals was accomplished by using the HMBC spectra [heteronuclear multiple bond connectivity: coupling of hydrogen and carbon-13 via two ($^2J_{CH}$) and three ($^3J_{CH}$) bonds]. For example, the starting point was the signal at δ_H 4.20 (s, 2H), assignable to 2H-24. The HMBC experiment established the identity of CH-3 (δ_C 79.50) as an oxygenated carbon; CH-5 (δ_C 55.20) distinguished by additional 3H-25 (δ_H 1.05) cross peak ($^3J_{CH}$); C-4 (δ_C 42.70) as the only quaternary carbon of the three and CH_3-23 (δ_C 22.90) as a methyl carbon. Proceeding along the molecule in this fashion, and using the hydrogens from only the six methyl resonances, allowed us to assign unambiguously the carbon atoms CH_2-1 (δ_C 44.00), CH_2-7 (δ_C 34.80), C-8 (δ_C 42.20), CH-9 (δ_C 45.20), C-10 (δ_C 37.50), C-13 (δ_C 91.40), C-14 (δ_C 43.20), CH_2-15 (δ_C 28.80), CH_2-19 (δ_C 39.90), C-20 (δ_C 31.80), CH_2-21 (δ_C 33.90), CH_2-24 (δ_C 65.30), CH_3-29 (δ_C 33.40) and CH_3-30 (δ_C 23.50). The quaternary carbon atoms C-8 (δ_C 42.20) and C-14 (δ_C 43.20) were distinguished by an additional H-18 (δ_H 2.03) cross peak for the latter carbon. Three methine (CH-2, CH-12 and CH-18), four methylene (CH_2-6, CH_2-11, CH_2-16 and CH_2-22) and two non-hydrogenated (C-17 and C-28) carbons showed no cross peaks with methyl hydrogens in the HMBC plot, as expected. Nevertheless, all they were unambiguously identified, CH-2 (δ_C 69.20) and CH-12 (δ_C 65.30) by correlations with 2H-1/H-3 and H-18, respectively. CH-18 (δ_C 51.80) was then distinguished by exclusion. The methylene carbons were assigned by use of a COSY technique. For example, the signals of H-5 (δ_H 1.15) and 2H-7 (δ_H 1.56 and 1.32) allowed the localization of 2H-6 (δ_H 1.66 and 1.56) hydrogens, which in turn led to assignment of CH_2-6 (δ_C 18.70) in the HMQC spectrum [heteronuclear multiple quantum coherence: spin–spin interaction of hydrogen and carbon-13 via one-bond ($^1J_{CH}$)]. In this way, the resonances of CH_2-11 (δ_C 29.60), CH_2-16 (δ_C 21.30) and CH_2-22 (δ_C 27.60) were also assigned. On the other hand, the HMBC plot was also used to assign C-17 (δ_C 43.00) on the basis of its connectivity with the signal corresponding to the H-16α (δ_H 1.30) hydrogen. C-28 (δ_C 178.80) was assigned on the basis of its characteristic chemical shift for carbonyl carbon.

Finally, the remaining methyl ^{13}C signals were determined from their connectivities with assigned methyl ^1H signals. In the HMBC spectrum, only the signal of 3H-25 (δ_H 1.05) showed a cross peak with the previously assigned CH_2-1 (δ_C 44.00); 3H-26 (δ_H 1.19) with CH_2-7 (δ_C 34.80) and 3H-27 (δ_H 1.38) with C-13 (δ_C 91.40) and CH_2-15 (δ_C 28.80). The assignments of the carbon signals were then carried out by an HMQC experiment (Table 1).

The assignment of CH_2 hydrogens as α or β was provided by analysis of splitting patterns (multiplicity and coupling constant) in the 1D NMR and especially $^1H \times {}^1H$-NOESY spectra (Table 1).

The NOESY data from Table 1 and ^{1}H–^{1}H splitting patterns were used to deduce the configuration and conformation of **1**, as illustrated in Fig. 2.

In conclusion, the complete ^{1}H and ^{13}C chemical shift assignment of the triterpenoid **1** (Table 1) has been achieved based on the combined use of 1D (^{1}H and ^{13}C) and 2D techniques (HMBC, HMQC, COSY and NOESY).

Halogenated compounds are not very widespread in nature and presumably in the plant the process that originates the chlorine atom should be mediated by appropriate enzymes.[9] The elemental composition $C_{36}H_{53}O_8Cl$ of **1** was determinate on the basis of high-resolution mass spectrometry. Its mass spectrum exhibited a molecular ion peak at *m/z* 649, $[MH]^+$, and a peak at *m/z* 651, $[MH + 2]^+$, in a ratio of 3 : 1.

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REFERENCES

1. N. R. Farnsworth, *Bioactive Compounds From Plants*. Wiley, Chichester (1990).
2. S. B. Mahato, A. K. Nandyand G. Roy, *Phytochemistry* **31**, 2199 (1992).
3. W. F. Reynolds, S. McLean, J. Poplawski, R. G. Enriquez, L. I. Escobar and I. Leon, *Tetrahedron* **42**, 3419 (1986).
4. D. Loganathan, G. K. Trivedi and K. V. R. Chary, *Magn. Reson. Chem.* **29**, 925 (1990).
5. E. F. Oliveira, Thesis, Universidade Federal do Ceará, Fortaleza, Brazil (1995).
6. F. J. A. Matos, *Farmácias Vivas*, 2nd ed. Universidade Federal do Ceará, Fortaleza (1991).
7. M. Muniz, *Ciência Hoje* **16** (96), 68 (1990).
8. F. J. Q. Monte, J. P. Kintzinger and R. Braz-Filho, *Magn. Reson. Chem.* **35**, 802 (1997).
9. S. Nittala, V. V. Velde, F. Frolow and D. Lavie, *Phytochemistry* **20**, 2547 (1981).

