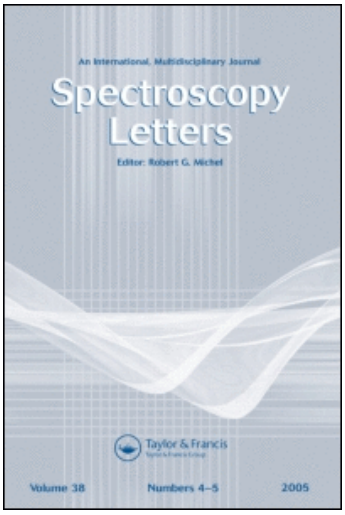

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

NMR Analysis of a Complex Spin System from a Siruo-Chamigrene

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To cite this Article Kaiser, Carlos R. , Pitombo, Liane F. and Pinto, Angelo C.(2000) 'NMR Analysis of a Complex Spin System from a Siruo-Chamigrene', Spectroscopy Letters, 33: 4, 457 — 467
To link to this Article: DOI: 10.1080/00387010009350130
URL: http://dx.doi.org/10.1080/00387010009350130

SPECTROSCOPY LETTERS, 33(4), 457–467 (2000)

NMR ANALYSIS OF A COMPLEX SPIN SYSTEM FROM A *SPIRO*-CHAMIGRENE

Keywords: 2,10-dibromo-3-chloro-8-hydroxy-β-chamigrene, marine molluscs, *Aplysia dactilomela*, ^{1}H and ^{13}C NMR, *gs*-2D NMR, AM1

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ABSTRACT

The compound 2,10-dibromo-3-chloro-8-hydroxy-β-chamigrene was analysed in detail by NMR Spectroscopy. The complete assignment of the signals in the ^{1}H and ^{13}C NMR spectra and the determination of the relative configurations were achieved by 2D NMR techniques, AM1 data and ^{1}H spectrum simulation. Comparisons of the results with related *spiro* chamigrene systems are also presented.

INTRODUCTION

Sesquiterpenes with the chamigrene skeleton are halogenated compounds that may also contain epoxide, hydroxyl, acetyl groups, as well as olefinic bonds. They are often found in marine algae and molluscs.[1] Marine natural products are of great interest in the process of drug discovery. Several secondary metabolites of molluscs from the genus *Aplysia* have been isolated, which show bactericide, fungicide, antitumoral, antileucemic, antineoplasic, citotoxic, citolitic and other activities. [2,3] Thus, knowing the stereochemistry is of primary importance for any compound that exhibits or may exhibit biological activity.

In this work we register the first occurence of 2,10-dibromo-3-chloro-8-hydroxy-β-chamigrene **1** in the marine mollusc *Aplysia dactilomela.*[4] In fact, **1** was isolated previously only from the red algae *Laurencia nipponica.* It was analysed by HRMS and ^{1}H (100 MHz) and ^{13}C (25 MHz) 1D-NMR but without signal assignments. The structure was elucidated mainly by chemical transformations. Very often, chamigrene structures have been elucidated in this way, a valid method, but laborious noting that quick and better results can be obtained by using 2D-NMR experiments. Thus, the purpose of this work is to present the full assignment of ^{1}H and ^{13}C spectra and stereochemical analysis of **1** by way of 2D-NMR experiments. Compound **1** is very suitable for this approach due to the problem in resolving a complex spin system that involves the C-1, C-2, C-4, C-5, C-8, C-9, C-10 hydrogens (eleven spins) with mostly overlapped or very close signals in the ^{1}H spectrum even at 300 MHz (see Fig. 3A). However, by way of 2D-NMR techniques such as: TOCSY,[6,7] NOESY,[6,7] and gradient selected COSY,[6,7] HMBC [6,7,10], and HSQC-ED [6-9,11] it was feasible to understand this complex spin system. The analysis was also followed by AM1 calculations. Simulation of the ^{1}H spectrum was performed for the final fitting of the chemical shifts and coupling constants.

Some comparisons are made with respect to the first analysis[5] of compound **1** and with a previous paper that dealt with related *spiro*-chamigrenes, dehydroxy prepacifenol epoxide **2**, and prepacifenol epoxide **3** that were also isolated from *Aplysia dactylomela.*[11]

RESULTS AND DISCUSSION

Compound **1** has the molecular formula $C_{15}H_{23}OBr_2Cl$ (by HRMS) and contains a double bond, as well as a hydroxyl group (by IR). The ^1H NMR spectrum (Fig. 3A) shows three methyl singlets (δ 1.72, 1.18, 0.91), multiplets between δ 2.7-2.0 (7H), three deshielded methyne signals between δ 4.8-4.4 (2xCHBr and CHOH), and two singlets at δ 5.47 and 5.07 due to an unsaturated exomethylene group. One of the methylene hydrogens and the hydroxyl signal are overlapped with the more deshielded methyl. The ^{13}C-Pendant[12] spectrum revealed the presence of three methyls (δ 24.52, 24.06, 17.55), five methylenes (δ 118.55, 45.56, 39.87, 39.85, 26.88), three methines (δ 76.70, 61.93, 60.42), and four quaternary carbons (δ 146.86, 72.24, 51.02, 44.02). Fig. 3A shows two signals around δ 4.7 that look to be two double doublets with small couplings, suggesting that they are due to hydrogens in *equatorial* positions. A more careful analysis, observing some cross signals that involve these hydrogens on expansion of the COSY, HSQC-ED and HMBC spectra (Fig. 1), revealed that the signals are two overlapped double doublets at δ 4.72 and δ 4.71, both having a large and a small coupling constant (c.a. 12.40/4.32 and 13.00/4.33 Hz). Thus, these hydrogens must occupy *axial* positions and also must be bonded to the brominated carbons (C-2 and C-8) thus accounting for their carbon chemical shifts δ 60.42 and δ 61.93 (see correlations in the expansion of the HSQC-ED spectrum in Fig. 1). The prior carbon chemical shifts are sufficiently distinct from the third deshielded methyne (C-10, δ_C

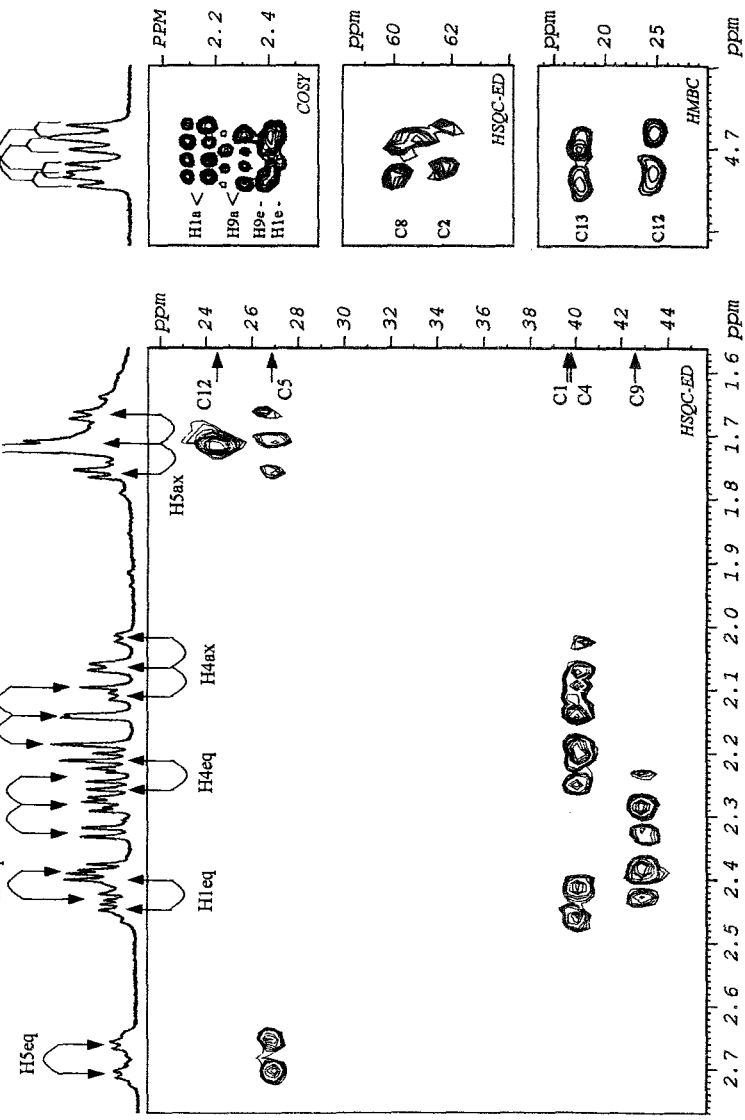

FIG. 1: Expansions of some 2D NMR spectra: left, HSQC-ED spectrum showing the methylene groups region (include also the C-12 methyl group) and right, COSY, HSQC-ED and HMBC spectra showing the methine CHBr groups region. (see text for more details)

TABLE 1

Summary of the observed HSQC-ED and HMBC correlations for compound **1**.[a]

δ_C \ δ_H	5.47	5.07	4.72	4.71	4.43	2.67	2.42	2.40	2.28	2.22	2.13	2.06	1.72	1.71	1.69	1.18	0.91
	15*s*	15*a*	8*ax*	2*ax*	10*eq*	5*eq*	1*eq*	9*eq*	9*ax*	4*eq*	1*ax*	4*ax*	12	5*ax*	OH	14	13
17.55			♦♦													♦♦♦	Θ
24.06																Θ	♦♦♦
24.52				♦♦						♦♦		♦	Θ				
26.88						Θ				♦♦		♦		Θ			
39.85				♦			Θ				Θ						
39.86				♦♦						Θ		Θ	♦♦♦				
42.56			♦♦		♦			Θ	Θ								
44.06							♦	♦	♦							♦♦♦	♦♦♦
51.02	♦♦♦	♦♦	♦		●					♦♦	♦♦			♦♦		♦♦♦	♦♦♦
60.42			Θ		♦			♦	♦♦							♦♦♦	♦♦♦
61.93				Θ							♦♦		♦♦♦				
72.24				♦♦						♦♦	●		♦♦♦				
76.70	♦♦	♦♦♦			Θ			♦									
118.55	Θ	Θ															
146.86	♦♦	♦♦						♦			♦♦♦			♦♦			

[a] *s* = *syn* to OH, *a* = *anti* to OH, *ax* = *axial*, *eq* = *equatorial*. HSQC-ED signals = Θ and HMBC signals with relative intensities[10] = ♦♦♦ (high), ♦♦ (medium) and ♦ (low). δ_C 44.06 (C-7), 51.02 (C-6), 72.24 (C-3) and 146.86 (C-11).

76.70) due to its carbinolic character (see Table 1 for HSQC-ED correlation), in which the hydrogen occupies an equatorial position (bdd, δ_H 4.43, J = 4.20 and 2.83 Hz).

With the aid of the COSY and TOCSY spectra it was feasible to assign several hydrogens to each ring (resumed on Fig. 3A). As previously observed for the two related *spiro* chamigrenes **2** and **3**,[11] compound **1** shows a long range coupling ("W" arrangment with c.a. $^4J_{H,H}$ = 3.80 Hz) between the H-1eq (δ 2.42) and the H-5eq (δ 2.67). The HSQC-ED experiment (expansion on Fig. 1) was more decisive in the analysis of the present spin system, particularly for the close or overlapped ^{1}H and ^{13}C methylene signals. As shown in Fig. 2, the broad double

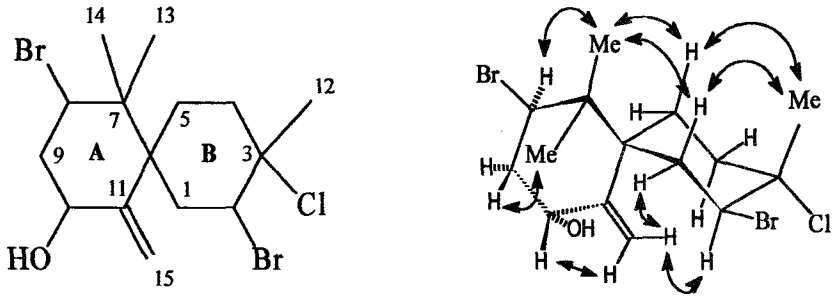

FIG. 2: Structure of compound **1** and main ^{1}H NOE interactions (indicated by arrows) observed in the NOESY spectrum.

triplet at δ_H 2.42 (H-1eq) is practically under the signal at δ_H 2.40 (ddd, H-9eq). The triple doublet at δ_H 1.71 (H-5ax) is overlapped by the broad hydroxyl signal (δ_H 1.69) and also by the methyl hydrogens at δ_H 1.72 (H-12). C-5 (δ_C 26.88) was readily assigned due to its hydrocarbon nature, as well as C-10 (δ_C 42.56) due to the previous COSY and TOCSY correlations (see Fig. 3A). However, only with further expansions of the HSQC-ED spectrum was it possible to distinguish C-1 (δ_C 39.85) from C-4 (δ_C 39.87).

With the aid of the HMBC spectrum it was feasible to confirm the location of three methyls in the molecule and to allow several assignments, mainly for quaternary carbons. A convenient way to resume the observed signals in the spectrum is presented in Table 1 (including the HSQC-ED relationships). Some significant $^{2\text{-}3}J_{C,H}$ cross signals observed in the HMBC spectrum can be pointed out: (a) H-13 with C-14 and H-14 with C-13 (ratifying correlations of H-13/H-14 with H-14/H-13 observed in the COSY and TOCSY spectra) confirming the *gem*-dimethyl group, (b) H-12 with C-4, C-2 and C-3 establishing the assignments of C-3 and C-12, (c) H-13 and H-14 with C-6 and C-7, H-15 with C-6 establishing the assignments of C-6

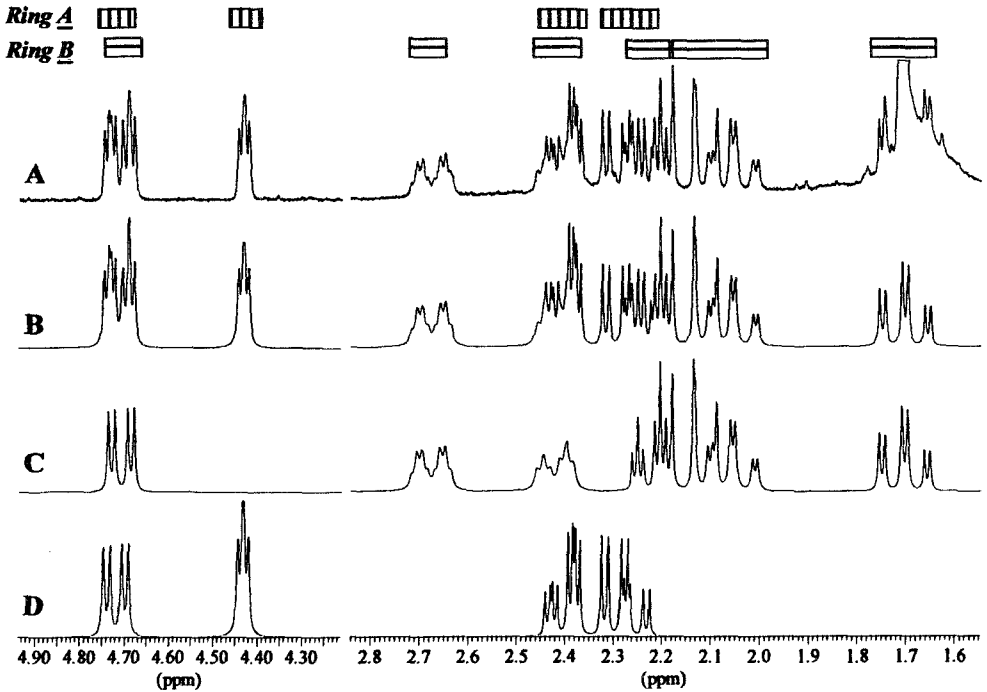

Fig. 3: ^{1}H NMR spectra: "1A" experimental, "1B" whole simulation (excluding singlets), "1C" simulation of ring B system, "1D" simulation of ring A system. Top of "1A" present the signals distinction between the two ring systems observed in COSY and TOCSY experiments (see text).

and C-7, (d) H-1ax, H-5ax, H-15 with C-11 confirming the assignment of this carbon.

All the NMR assignments were corroborated by the AM1 data which indicates that both rings have chair conformations with some distortions mainly for ring A due to the presence of an sp^2 carbon. This result is easily observed by comparing the *diaxial* dihedral angles between the pairs H-2ax/H-1ax (ϕ = 174.24°), H-4ax/H-5ax (ϕ = 178.76°) and H-8ax/H-9ax (ϕ = 170.62°) that correlated well with the corresponding $^3J_{H,H}$ coupling constants 13.10 Hz, 13.90 Hz and 12.40 Hz. The NOESY experiment confirmed all NMR and AM1 data with respect to the stereochemistry of both rings as shown in Fig. 2 (some of the main NOE correlactions are presented). The results from the NMR analysis of compound **1** lead to a good fit of the experimental and simulated spectra (Fig. 3).

The summary of the chemical shifts and coupling constants are shown in Table 2 revealing that the previous analysis of the ^1H and ^{13}C assignments was inaccurate.[5] It is more interesting is to compare the present data for compound **1** with the previous work involving two related *spiro* chamigrenes **2** and **3**.[11] The most significant changes in δ_C comparing dehydoxyprepacifenol epoxide **2** with compound **1** are for C-6 (47.92 ↔ 51.02), C-13 (22.11 ↔ 17.55) and C-14 (26.66 ↔ 24.06). The deshielding of C-6 in compound **1** can be attributed to the presence of the vicinal sp^2 carbon (C-11). The deshieldings of C-13 and C-14 in compound **2** can be attributed to steric effects due to the presence of an axial methyl group (C-15) and also due to crowding by the bromine (C-8) caused by the *exo* epoxide ring (C-8/C-9). In fact, the corresponding H-13 and H-14 are also more deshielded in compound **2** than in compound **1** due to the same steric effects (H-13, δ_H 0.98 ↔ 0.91 and H-14, δ_H 1.37 ↔ 1.18).[13] The steric effect of the H-15*anti*(OH) could be the main factor that leads to a

TABLE 2

Summary of the ^{1}H and ^{13}C chemical shifts and ^{1}H coupling constants from compound **1**, including comparison with the other one analysis.[5]

C	H	δ_C	δ_H (*J*/Hz)	δ_C[5]	δ_H (*J*/Hz)[5]
1	**ax**	39.85	2.13 dd (14.20, 13.10)	26.7, 39.6,	2.0-2.5 m
	eq		2.42 bdt (14.20, 4.33, 3.80)	39.6, 42.4	
2	**ax**	61.93	4.71 dd (13.10, 4.33)	60.1, 61.6	4.73 dd (13, 4), 4.74 dd (11, 5.5)
3	--	72.24	--	71.9	--
4	**ax**	39.87	2.06 btd (13.93, 13.90, 2.96)	26.7, 39.6,	2.0-2.5 m
	eq		2.22 dt (13.93, 3.40, 3.38)	39.6, 42.4	
5	**ax**	26.88	1.71 td (14.30, 13.90, 3.38)	26.7, 39.6,	2.0-2.5 m
	eq		2.67 bdq (14.30, 3.80, 3.40, 2.96)	39.6, 42.4	
6	--	51.02	--	43.8, 50.8	--
7	--	44.06	--	43.8, 50.8	--
8	**ax**	60.42	4.72 dd (12.40, 4.32)	60.1, 61.6	4.73 dd (13, 4), 4.74 dd (11, 5.5)
9	**ax**	42.56	2.28 ddd (14.08, 12.40, 4.20)	26.7, 39.6,	2.0-2.5 m
	eq		2.40 ddd (14.08, 4.32, 2.83)	39.6, 42.4	
10	**OH**	76.70	1.69 bs	76.0	2.0-2.5 m
	eq		4.43 bdd (4.20, 2.83)		4.43 dd (3, 3)
11	--	146.86	--	146.50	--
12	**12**	24.52	1.72 s	17.4, 23.9, 24.3	0.91 s, 1.17 s, 1.71 s
13	**13**	17.55	0.91 s	17.4, 23.9, 24.3	0.91 s, 1.17 s, 1.71 s
14	**14**	24.06	1.18 s	17.4, 23.9, 24.3	0.91 s, 1.17 s, 1.71 s
15	***anti*(OH)**	118.55	5.07 s	118.1	5.06 s, 5.46 s
	***syn*(OH)**		5.47 s		

deshielding of H-1eq and H-2ax for compound **1** with respect to compound **2** (2.41 ↔ 2.23 and 4.59 ↔ 4.71). The shielding of the H-5eq from compound **2** in relation to compound **1** is probably due to the absence of the anisotropic effect of the *endo* epoxide (C-10/C-11).[14] Prepacifenol epoxide **3** and compounds **2** and **1** show a measurable and increasing $^{4}J_{H,H}$ between H-1eq and H-5eq (2.32, 3.65, and 3.80). The same trend was observed for

the *diaxial* dihedral coupling constants of the H-1ax/H-2ax and H-4ax/H-5ax pairs in compounds **1** and **2** (13.10 ↔ 13.00 and 13.90 ↔ 12.42). This suggests that compound **1** possesses the more perfect chair conformation in ring B with respect to compounds **2** and **3**.

EXPERIMENTAL

Compound **1** was isolated from *Aplysia dactylomela* as described previously.[4] The NMR spectra were recorded using 10 mg/ml $CDCl_3$ solutions on a Bruker DRX300 spectrometer equipped with a three axis gradient unity and an inverse multinuclear probe at 300K. Tetramethylsilane was used as an internal reference for the chemical shifts. The XwinNmr 1.3 pulse/processing programs were used for the spectra and the WinDaisy 2.0 and WinNmr 5.1 programs for the ^1H simulations from Bruker GMBH, and PC Spartan plus for the AM1 calculations from Wavefunction, Inc.

The 1D spectra were acquired at 32K and 64K data points and the 2D spectra using 1024x256 data point matrices, with a spectral width of 2000 and 10000 Hz for the ^1H and ^{13}C, respectively. The number of scans as 16, 32, 32, 32 and 64 were used for *gs*-COSY, TOCSY, HSQC-ED, NOESY and *gs*-HMBC, respectively. Spin-lock of 2.5 ms/40 loops was used for TOCSY and a mixing time of 800 ms was used for NOESY. Echo/anti-echo FT mode was used for the HSQC-ED. Zero filling and/or linear prediction were used in all 2D experiments.

ACKNOWLEDGMENTS

We thank NPPN (UFRJ) for the HRMS analysis and CNPq, FAPERJ, CAPES, PADCT-FINEP and FUJB for financial support.

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Date Received: November 22, 1999
Date Accepted: March 10, 2000