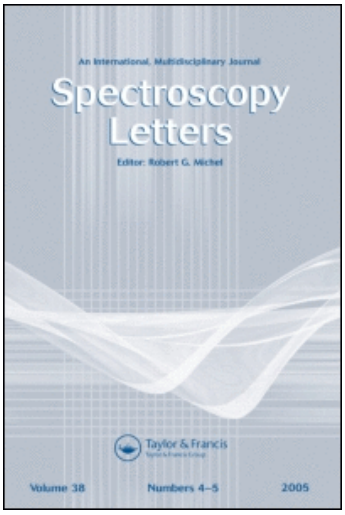

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C-13 and H-1 NMR Assignments of the Chamigrenes Prepacifenol and Dehydroxyprepacifenol Epoxioes

Carlos R. Kaiser[a]; Liane F. Pitombo[a]; Angelo C. Pinto[a]
[a] Institute de Qulmica-DQO, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, RJ, BRASIL

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C-13 and H-1 NMR ASSIGNMENTS OF THE CHAMIGRENES PREPACIFENOL AND DEHYDROXYPREPACIFENOL EPOXIDES

Keywords: chamigrenes, prepacifenol epoxide, dehydroxyprepacifenol epoxide, ^{1}H and ^{13}C NMR, COSY, NOE difference, HMBC/GS, HSQC-TOCSY/GS, HSQC-DE/GS.

Carlos R. Kaiser *, Liane F. Pitombo and Angelo C. Pinto

Instituto de Química-DQO, Universidade Federal do Rio de Janeiro, Ilha do Fundão, CT, Bloco A, 6° andar, 21949-900 Rio de Janeiro-RJ, BRASIL

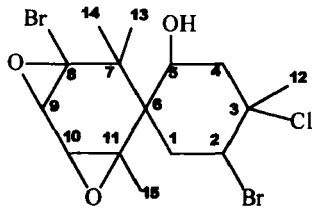
ABSTRACT

A complete assignment of the signals in the ^{1}H and ^{13}C NMR spectra and the stereostructural analysis of the compounds prepacifenol epoxide and the new dehydroxyprepacifenol epoxide are presented. These compounds were extracted from the marine mollusc *Aplysia dactylomela* and represent the first occurrence of chamigrenes found in Brazilian waters. The NMR analyses are supported by NOE difference and COSY experiments and also by gradient selected HMBC, HSQC-TOCSY and HSQC-DE. ^{1}H spectra simulations were done for the final fitting of the chemical shifts and coupling constants.

INTRODUCTION

Aplysia dactylomela is a marine mollusc that lacks protection by an external shell. This mollusc exudes some distasteful components in a

mucus from the mantle in order to deter potential predators. The general assumpsion is that these molluscs accumulate metabolites, such as chamigrene type sesquiterpenes, from algae through their food chain.[1] In fact, prepacifenol epoxide 1 was first isolated from the marine red algae *Laurencia pacifica* and analysed by means of Mass and ^{1}H NMR spectrometries.[2] However, the structure of this halogenated sesquiterpene diepoxide has not been investigated in detail, principally with respect to the stereochemistry of ring **B** (which contains two halogen atoms). We have isolated compound 1 and a related structural analogue which has not yet been described, from the visceral extracts of the *Aplysia dactylomela*.[3] This is the first occurrence of chamigrenes in Brazilian waters and the new compound is named here dehydroxyprepacifenol epoxide 2.



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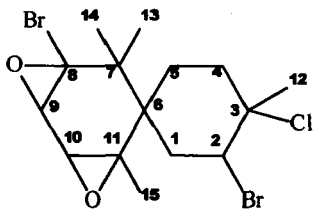

2

The characterization of 2 and the assignment of all signals in the ^{1}H and ^{13}C NMR spectra of compounds 1 and 2 were done with the aid of NOE-difference[4] and COSY[5] spectra. The analysis is also supported by pulse field gradient techniques such as HMBC/GS (gradient-selected HMBC), HSQC-TOCSY/GS (gradient-selected HSQC including also the HOHAHA correlactions) and the HSQC-DE/GS (gradient-selected

HSQC with the cross signals edited as a DEPT).[6-10] AM1 data and ^1H spectra simulations were also used for the determination of the stereostructures of the chamigrenes **1** and **2**.

RESULTS AND DISCUSSION

Analysis of compound **2** by HRMS lead to the molecular formula $C_{15}H_{21}O_2ClBr_2$. The ^{13}C NMR spectrum does not show any double bond because all peaks appear between δ 77-20. The ^1H NMR spectrum shows two methyne protons with the characteristic chemical shifts of epoxides at δ 3.09 and 3.64 and no couplings thus suggesting that they belong to different epoxide rings. In addition, the other methyne proton at δ 4.59 must be bonded to a carbon that contains one of the halogen atoms. From the fifteen carbon atoms present, we saw by way of the HSQC-DE experiment (Fig. 1) that four of them are methyl, three are methylene and three are methyne ones (beside an impurity, present at δ 1.27). Thus, the other five are non protonated carbons. The above foregoing analysis of compound **2** showed this structure to be a spiro diepoxy sesquiterpene related to the previously isolated prepacifenol epoxide **1**.[3] The difference between the two compounds being the presence of an additional hydroxyl group.

The four methyl carbons in compound **2** must be bonded to non protonated carbons because they are all singlets in the ^1H spectrum. The ^1H chemical shifts do not show methylene carbons bonded directly to a heteroatom but indicate that two of them are close to carbons bonded to heteroatoms. Furthermore, the spectrum exhibited several overlapped signals.

The HOHAHA connectivities in the HSQC-TOCSY experiment (Fig. 2) shows three spin systems that, with the HSQC correlactions,

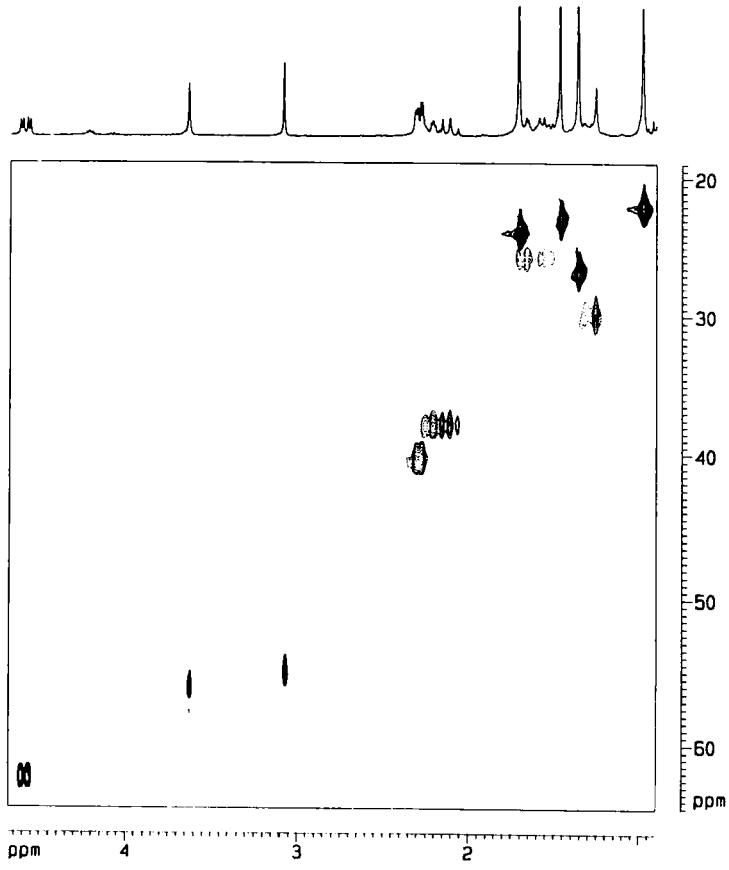

FIG. 1: HSQC-DE spectrum of compound **2** (solid lines for CH_3 and CH and dotted lines for CH_2)

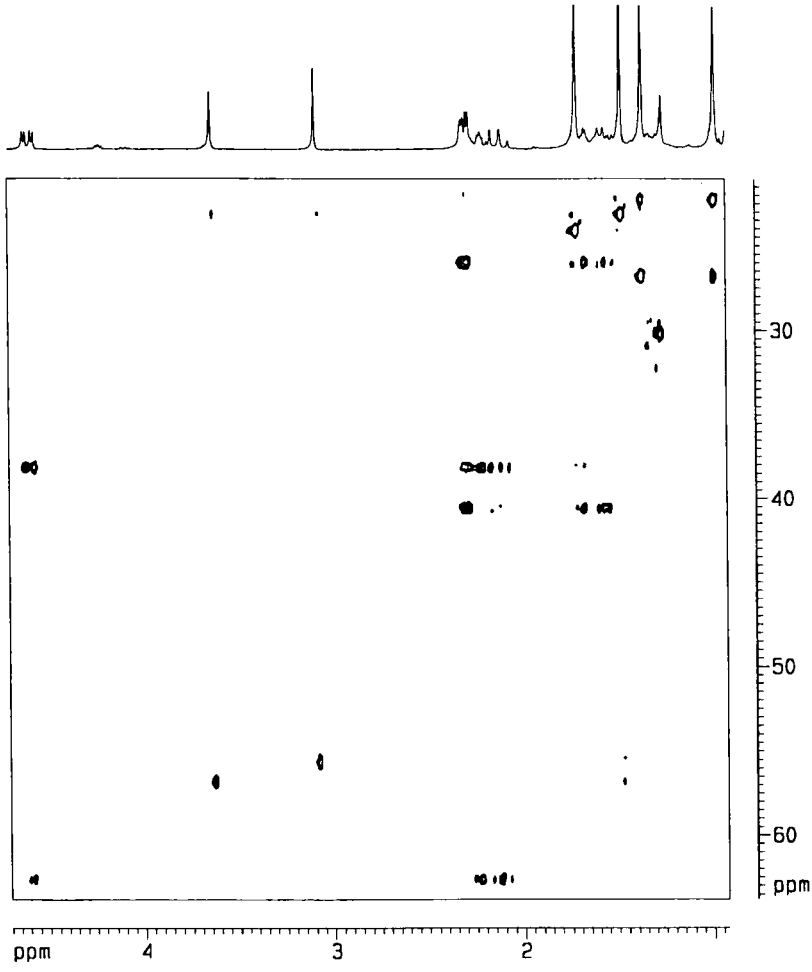

FIG. 2: HSQC-TOCSY spectrum of compound **2**

lead to the characterisation of three structural fragments for compound **2**. In summary: a) for the {$(CH_3)_2C$} fragment the two most shielded methyls in the ^1H spectrum exhibits mutual HOHAHA cross signals; b) in the {C-[O]-CH-CH-C-[O]-C-CH_3} fragment the lack of a coupling and cross signal between the epoxide protons and also the presence of correlations with the methyl protons at δ 1.48 suggest that these protons are close but in a *trans* epoxide ring arrangement (C-[O]-C denotes an epoxide ring); c) the several HOHAHA connectivities involving the most deshielded methyne and the three methylene groups suggest the presence of a six membered ring formed by the {C-CH_2-CHBr-C-CH_2-CH_2} fragment. In the later, the TOCSY connectivities between the CH_2CHBr and CH_2CH_2 groups are not as evident because of the very low intensities of the cross signals. Nevertheless, the COSY experiment shows a cross signal between one of the C-1 and one of the C-5 protons (δ 2.23 and 1.55) which suggests a perfect "W" arrangement that justifies the c.a. 3.65 Hz coupling constant and the broad double-triplet pattern for this H-1 in the ^1H spectrum. Other cross signals, due to long range couplings, that are detected by the COSY experiment involve H-2 with H-12 (δ 4.59 and 1.72), H-13 with H-14 (δ 0.98 and 1.37) and both the epoxide protons with H-15 (as in the TOCSY).

The HMBC confirms that the two most shielded methyls in the ^1H spectrum are bonded to the same carbon due to the mutual three bond C/H cross signals. Other detected long range C-H cross signals of importance to confirm the structure of compound **2** are: H-15 with C-10 and C-11; H-13/H14 with C-6, C-7 and C-8; H-12 with C-2, C-3 and C-4; H-10 with C-8, C-9 and C-11; H-9 with C-8, C-10 and C-11; C-5 protons with C-4 and C-11; C-4 protons with C-2, C-3, C-5, C-6 and C-12; H-2 with C-1, C-3 and C-12; C-1 protons with C-2, C-6 and C-11. The latter

correlations do not help to determine the stereostructure of **2** but were very helpful in discerning between C-9 and C-10 (δ 56.54 and 55.33), C-3 and C-8 (δ 71.28 and 76.17), C-6 and C-7 (δ 47.92 and 45.59), C-1 and C-4 (δ 37.86 and 40.33).

Compound **2** could exhibit one of eight possible conformations but molecular structure models and AM1 analyses can eliminate six of them due to severe steric crowdings. This left only two possibilities either where C-12 is *axially* oriented in a chair conformation or where it's *equatorially* oriented in a boat conformation with the C-7 at the upper site of the C-6/C-8 to C-11 plane for both. Only the chair conformation can justify the observed H-2 vicinal coupling constant of c.a. 13.11 Hz (a diaxial arrangement with H-1_{ax}). The NOE difference experiments performed on compound **2** confirm the latter observations. Figure 3 illustrates, beside a summary of all experimental results, that the irradiation of H-13 (*syn* with respect to C-15) at δ 0.98 gives enhancements at H-1_{eq} (δ 2.23) and H-10 (δ 3.09); irradiation of H-14 (*anti* with respect to C-15) at δ 1.37 gives enhancement at H-1_{ax} (δ 2.12); irradiation of H-15 gives enhancements at H-10 (δ 3.09) and H-2 (δ 4.59).

Prepacifenol epoxide **1**, whose molecular formula is $C_{15}H_{21}O_3ClBr_2$, was analysed in the same way as the related dehydroxy **2**. The COSY spectrum shows that the cross signal due to the long range coupling (now c.a 2.32 Hz) between H-1_{eq} and H-5_{eq} (now at δ 3.98) is still present. Thus, the hydroxy group is at the C-5 *axial* position (giving a vicinal coupling with H-5_{eq} of c.a. 5.9 Hz) , although it does not change the stereochemistry of the **A** and **B** rings established for the dehydroxy compound **2**. In fact the presence of this hydroxyl affects the chemical shifts of the neighbouring hydrogens and carbons either

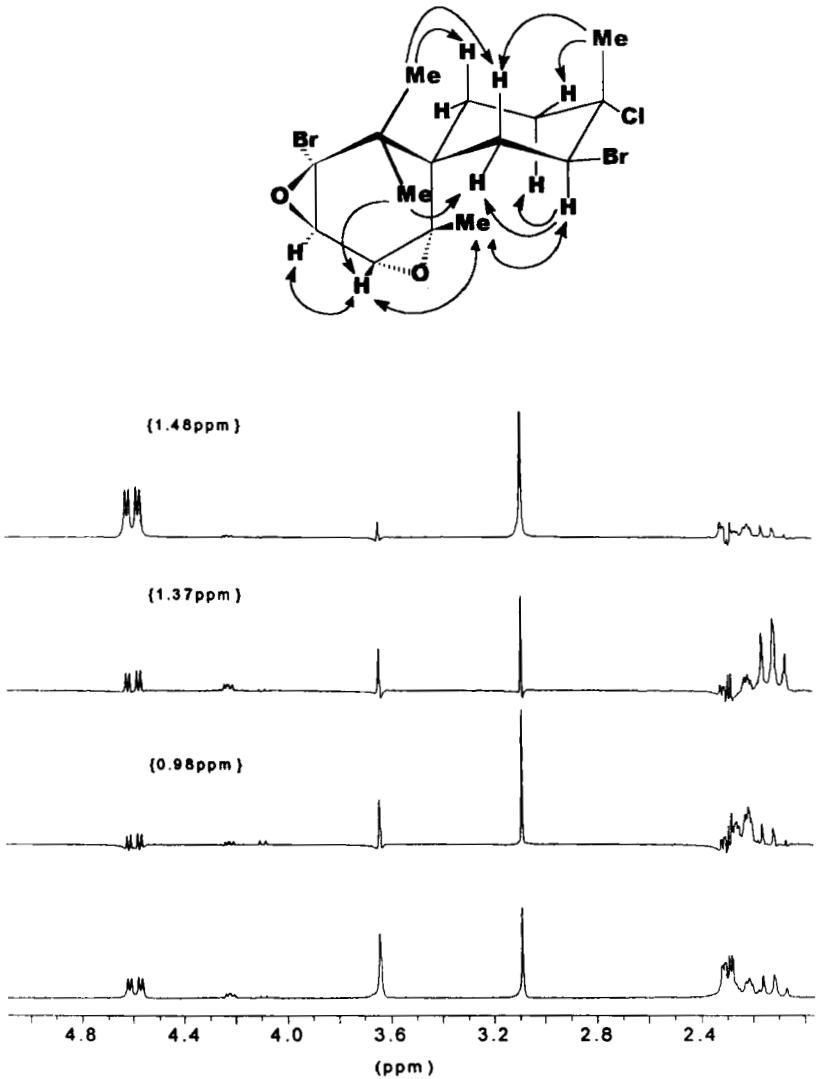

FIG. 3: NOE observed and some difference spectra of compound 2

spacially or through the bonds, due to several mechanisms involving electronic and steric effects.[11,12] For example, a comparison of the changes in the chemical shifts on going from **2** to **1** shows: a deshielding due to the electric field and inductive effects at C-4 (from δ 40.33 to 47.08), C-6 (from δ 47.92 to 50.04), H-4_{eq} (from δ 2.29 to 2.47), H-4_{ax} (from δ 2.30 to 2.47); a deshielding due mainly to steric effects at C-12 and H-12 (from δ 23.94 to 27.95 and from δ 1.72 to 1.88), C-14 and H-14 (from δ 26.66 to 27.08 and δ 1.37 to 1.45); a dipole-induced charge polarization on H-1_{ax} that gives a deshielding (from δ 2.12 to 2.48) with the counterpart shielding on C-1 and H-1_{eq} (from δ 37.86 to 33.80 and from δ 2.23 to 2.10).

Under mildly acidic conditions prepacifenol epoxide **2** can be converted to Johnstonol.[2,3] A X-ray crystallography experiment of a perfect colourless orthorhombic crystal of Johnstonol revealed that the chlorine atom is exactly at the proposed C-3 position and not at C-2 or C-8, the other possible positions.

Finally, the ^{1}H spectra simulations were very helpful for resolving the signals mainly for the overlapped ones. The results are illustrated in Fig. 4 and Fig. 5 for the dehydroxyprepacifenol epoxide **2** and the prepacifenol epoxide **1**, respectively. Table 1 summarizes the chemical shifts and coupling constant data for both compounds.

EXPERIMENTAL

Compounds **1** and **2** were isolated from *Aplysia dactylomela* as described before.[3]

The NMR spectra were recorded using 18 mg/ml $CDCl_3$ solutions for compounds **1** and **2** on a Bruker DRX300 spectrometer equiped with a three axis gradient unity and an inverse multinuclear probe at 300K.

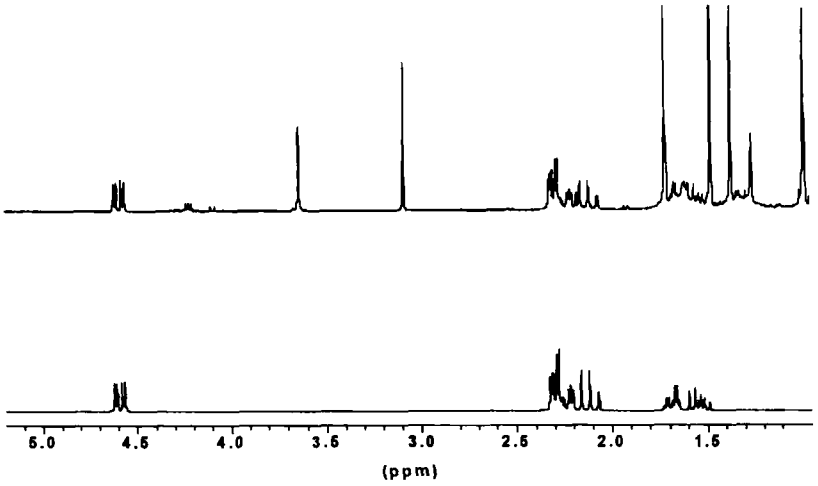

FIG. 4: Experimental and simulated spectra of compound **2**

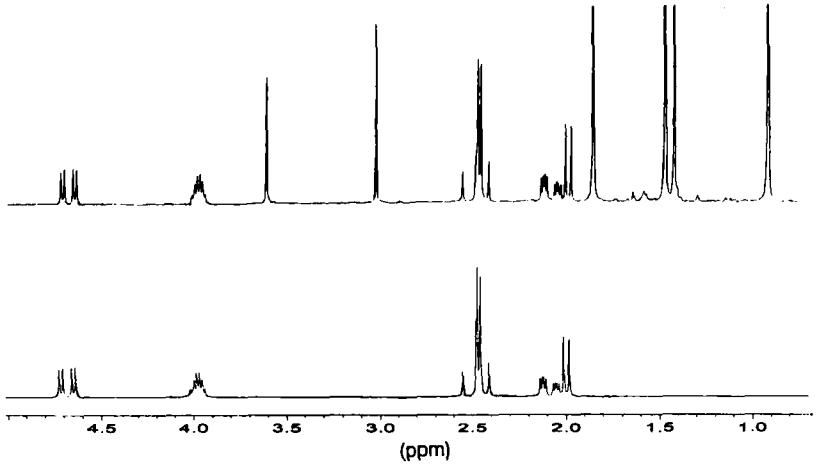

FIG. 5: Experimental and simulated spectra of compound **1**

TABLE 1

^{1}H and ^{13}C NMR Chemical Shifts and ^{1}H Coupling Constants from Prepacifenol Epoxide **1** and Dehydroxyprepacifenol Epoxide **2**

		← PE 1 →		← DPE 2 →	
C	H	δ_C	δ_H (J/Hz)	δ_C	δ_H (J/Hz)
1	ax	33.80	2.48 t (14.01, 13.83)	37.86	2.12 dd (14.00, 13.11)
	eq		2.10 ddd (14.01, 3.95,2.32)		2.23 dt (14.00,4.22, 3.65)
2	ax	62.06	4.69 dd (13.83, 3.95)	62.52	4.59 dd (13.11, 4.22)
3	–	71.47	–	71.28	–
4	ax	47.08	2.47 d (3.40)	40.33	2.30 m
	eq		2.47 d (3.31)		2.29 m
5	OH	69.69	1.99 d (5.90)	25.75	–
	ax		–		1.68 dq (14.25, 3.90,3.70,3.65)
	eq		3.98 dq (5.90,3.40,3.31,2.32)		1.55 dt (14.25, 12.42,5.85)
6	–	50.04	–	47.92	–
7	–	46.88	–	45.59	–
8	–	75.40	–	76.17	–
9	endo	55.49	3.62 s	56.54	3.64 s
10	exo	56.53	3.06 s	55.33	3.09 s
11	–	60.91	–	59.99	–
12	12	27.95	1.88 s	23.94	1.72 s
13	13	24.26	0.95 s	22.11	0.98 s
14	14	27.08	1.45 s	26.66	1.37 s
15	15	22.07	1.48 s	22.97	1.48 s

Tetramethylsilane was used as internal reference for the chemical shifts. The Xwin-Nmr 1.3 pulse programs were used for the spectra and the WinDaisy 2.0 and WinNmr 5.1 programs for the ^{1}H simulations, licenced by Bruker.

The 1D spectra were acquired at 32K and 64K data points with a spectral width of 3000 and 15000 Hz for the ^{1}H and ^{13}C respectively. For all the 2D analyses the spectral width 2700 and 9000 Hz and 1024x256 data points matrixes were used with 16, 112, 64 and 64 scans for the COSY, HMBC, HSQC-DE, HSQC-TOCSY respectively. A 176.9 ms spin-lock was used for the last one. Echo/anti-echo FT mode were used for the last two. Sine for the COSY and cosine-squared window functions for the other three were also used.

ACKNOWLEDGEMENTS

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REFERENCES

1. Faulkner D. J. *Marine Natural Products: Metabolites of Marine Invertebrates,* Nat. Prod. Rep. **1984**, 1: 551-598.

2. Faulkner D. J., Stallard M. O., Ireland C. *Prepacifenol Epoxide, A Halogenated Sesquiterpene Diepoxide*, Tetrahedron Lett. **1974**, 40 : 3571-3574.

3. Pitombo L. F., Kaiser C. R., Pinto A. C. *Occurence of Chamigrenes in Aplysia Dactilomela from Brazilian Waters*, Bol. Soc. Chil. Quím. **1996**, 41: 433-436.

4. Sanders J. K. M., Mersh J. D. *Nuclear Magnetic Double Resonance: The Use of Difference Spectroscopy*, Prog. Nucl. Magn. Reson. Spectrosc. **1982**, 15: 353-400.

5. Nagayma K., Kumar A., Wuethrich K., Ernst R. R. *Experimental Techniques of Two-Dimensional Correlated Spectroscopy*, J. Magn. Reson. **1980**, 40: 321-334.

6. Willker W., Leibfritz D., Kerssebaum R., Bermel W. *Gradient Selection in Inverse Heteronuclear Correlation Spectroscopy*, Magn. Reson. Chem. **1993**, 31: 287-292.

7. Croasmun W. R., Carlson R. M. K. (Eds.) *Two-Dimensional NMR Spectroscopy*, 2nd ed. New York: VCH, **1994**.

8. Braum S., Kalinowski H.-O., Berger S. *100 and More Basic NMR Experiments,* Weinheim: VCH, **1996**.

9. Domke T. *A New Method to Distinguish Between Direct and Remote Signals in Proton-Relayed X,H Correlations*, J. Magn. Reson. **1991**, 95: 174-177.

10. Martin G. E., Spitzer T. D., Crouch R. C., Luo J.-K., Castle R. N. *Inverted and Suppressed Direct Response HMQC-TOCSY Spectra - A Convenient Method of Spectral Editing*, J. Heterocyclic Chem. **1992**, 29: 577-582.

11. Kaiser C. R., Rittner R., Basso E. A. *Substituent-Induced ^1H Chemical Shifts of 3-Substituted Camphors*, Magn. Reson. Chem. **1997**, 37: in press.

12. Basso E. A., Kaiser C. R., Rittner R., Lambert J. B. *Electronic Interactions Implied by the Non-Additivity of Carbon-13 Substituent Parameters in 2-Substituted Cyclohexanones*, Magn. Reson. Chem. **1994**, 32: 205-209.

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