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Artigo

COMPLETE ^{1}H AND ^{13}C NMR STRUCTURAL ASSIGNMENTS FOR A GROUP OF FOUR GOYAZENSOLIDE-TYPE FURANOHELIANGOLIDES[#]

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Four goyazensolide-type sesquiterpene lactones – lychnofolide, centratherin, goyazensolide and goyazensolide acetate – were thoroughly studied by NMR experimental techniques. ^{1}H NMR, ^{13}C NMR {^{1}H}, COSY, HMQC, HMBC, *J-res.* and NOE experiments were performed to provide the needed structural information. Complete and unequivocal assignment, including the determination of all multiplicities, was obtained for each structure and the data collections are presented in tables.

Keywords: sesquiterpene lactones; furanoheliangolides; complete NMR assignments.

INTRODUCTION

Sesquiterpene lactones (SL) constitute an important class of natural products (NP) that display numerous biological activities such as antibacterial, antifungal, cytotoxic, anti-tumoral, anti-inflammatory, anti-parasitic actions, etc.[1-5] Their structures show significant variety and complexity, making structural elucidation a challenge. Since the first articles on isolation and identification of SL were published in the early 1960s, some published data has proven incomplete, inaccurate or even wrong, due to equipment limitations at the time.[6]

The need for structural elucidation has emerged since the beginning of organic chemistry and remains a challenge, both in synthesis as well as in NP chemistry. Nuclear Magnetic Resonance (NMR) is now widely regarded as an indispensable technique for the structural elucidation of NP, as well as of synthetic organic chemicals. This is a technique offering increasing potential due to the continuous evolution of the NMR equipment and the development of new techniques, such as the 2D NMR, which have enabled much more detailed, complete, and reliable analysis of substances with complex structures, in comparison to decades earlier.

Therefore, it has become attractive and even necessary to conduct a study of several SL toward a full investigation of the structures with unambiguous assignments of ^{1}H and ^{13}C NMR data. Complete assignments data available in the literature can be highly useful for the assignment of the same or similar structures isolated in future works. Further to our research group interest on sesquiterpene lactones,[1,8-10] we present this study of four furanoheliangolides: lychnofolide (**1**); centratherin (**2**); goyazensolide (**3**) and goyazensolide acetate (**4**) (Figure 1). All substances were thoroughly structurally studied by NMR experiments including ^{1}H NMR, ^{13}C NMR {^{1}H}, COSY, HMQC and HMBC.

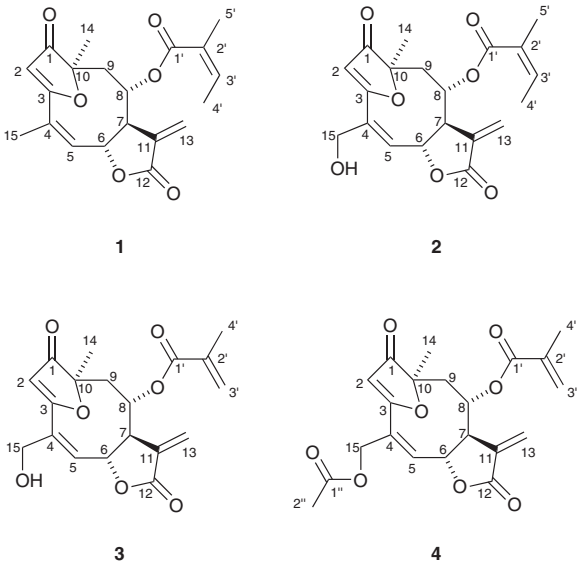

Figure 1. *Structure of the sesquiterpene lactones*

EXPERIMENTAL

Sample obtention

The SLs lychnofolide (**1**), centratherin (**2**) and goyazensolide (**3**) were all isolated as described in previous studies performed by our research group,[8-10] while goyazensolide acetate (**4**) was prepared from a sample of **3**, as prepared previously in one of our earlier studies on SL structural modifications.[11,12]

NMR measurements

For the measurements, sample concentrations were kept within

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[#]Artigo em homenagem ao Prof. Otto R. Gottlieb (31/8/1920-19/6/2011)

a 20-25 mg mL^{-1} concentration range in $CDCl_3$ with 0.03% TMS. Sample temperature was 300 K.

NMR spectra were performed on a Bruker Avance DRX500 spectrometer at 500.13 MHz for ^1H and 125.76 MHz for ^{13}C in which a 5 mm inverse probe head (BBI 1H-BB) was installed. Some ^{13}C NMR spectra were also recorded on a Bruker Avance DRX400 spectrometer operating at 400.13 MHz for ^1H and 100.61 MHz for ^{13}C, with a direct probe head of 5 mm (DUL 13C-1). The ^1H NMR spectra were acquired with spectral width = 5.48 kHz; 64k data points; 16 scans – providing digital resolution of 0.083 Hz (^1H 30° pulse width = 8.5 μs). For ^{13}C, spectral width = 23.98 kHz; 32k data points and 1024 scans were used – providing digital resolution of 0.732 Hz (^{13}C 30° pulse width = 14.25 μs). DEPT 135 (512 scans) and 2D chemical shift correlation experiments were performed using standard pulse sequences supplied by the spectrometer manufacturer. Long-range $^{13}C/^1H$ chemical shift correlations were obtained in experiments with delay values optimized for $^2J(C, H)$ = 8 Hz.

RESULTS AND DISCUSSION

Initial examination of all SL ^1H NMR spectra showed that groups of signals rarely overlapped, allowing a detailed analysis of multiplicity. However, the complexity of some signal couplings hampered the process of clarifying multiplicity. In some cases, only the use of *J-resolved* experiments proved able to determine multiplicity. 2D experiments allowed the unequivocal assignments of ^{13}C NMR shifts.

Despite the fact that a spectral assignment study has been published on goyazensolide (**3**),[13] the data in the cited paper failed to include experimental *J* values or 2D NMR data. In addition, some *J* values were missing (such as *J*(3',4'a) and *J*(3',4'b)) and some differed from the experimental values (such as *J*(5,15a) and *J*(5,15b)). We thus decided to include all the assignments for **3** in the current study.

Initially, the ^1H NMR spectrum of lychnofolide (**1**) was studied in detail and multiplicity was easily determined for most signals. The scattering of signals across the scale provided simple assignment. The multiplicities of the signals of H5, H6 and H7 required further effort to be clarified. This was undertaken by comparison of the experimental signals with those calculated by the computer program FOMSC3.[14] In this program, values for spin couplings that can be measured in *J-res* experiments are entered and the program then simulates the signal. Thus, comparison of the signal appearance and the shift for each peak with the experimental signal can lead to conclusions regarding the values employed: considerable appearance similarity and good similarity of each peak's chemical shift proves the reliability of the measured *J* values. Therefore, even these complex signals had their multiplicity clarified. Figure 2 shows the comparison of experimental and simulated H7 signals for **1**. Notably, the difference in each peak chemical shifts for any pair of experimental and simulated peak is no higher than 0.1 Hz. Moreover, the experimental and calculated signal shapes are very similar.

This kind of multiplicity cannot be clarified by ^1H NMR spectrum processing alone. The use of *J-res* experiments and the simulations assure these values and correct multiplicity. The complete ^1H NMR data for **1** are given in Table 1.

For the study of centratherin (**2**), the greatest challenge was the multiplicity clarification of the H15 signal and measurement of all coupling constants involved. First impressions led us to consider only the central part of the signal, since the other peaks presented very low intensity (Figure 3), but this proved to be incorrect. More careful observation revealed that H15a and H15b have different chemical shifts and the smaller peaks are part of the signal, showing a *J* value of approximately 15Hz, which is expected for geminal hydrogens. Due to the presence of second order interactions, the signals were

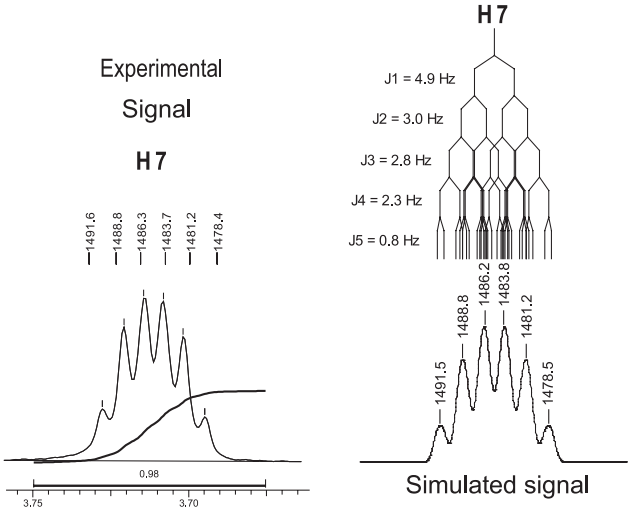


Figure 2. *Comparison of experimental and simulated H7 signals for **1***

Table 1. Some experimental and calculated *J* values (in Hz) for lychnofolide (**1**)

J	Calculated	Experimental
J (8,9α)	11.61	11.8
J (8,9β)	2.87	2.0

subsequently simulated using a program that takes into account these interactions: SimEsp_NMR,[15] using the *J* values from Table 2. Once again the similarity between experimental and simulated signals confirmed multiplicity and measured *J* values.

The signal also presents four coupling constants for each hydrogen. Since both of these are sufficiently close to couple only with two other hydrogens (H5 and H6), the complex multiplicity remains unexplained. Only the presence of an apparent triplet near 2.35 ppm resolved this problem. This is the hydrogen signal from the –OH group in position 15. This doublet of doublets presents coupling constants of 6.1 and 6.3 Hz, explaining the signals shown in Figure 3. The signal for each H15 was found to be a fourfold doublet. All ^1H NMR data for centratherin are shown in Table 3.

For goyazensolide (**3**), the main difference in comparison to structures **1** and **2**, was the ester side chain at position 8. This gives an methacrylate instead of an angelate. This causes the presence of two olefinic hydrogen signals for H3'a and H3'b. The signal of H3'b is a clear and isolated doublet of quartets, but the signal of H3'a is partially overlapped with the signal of H5, rendering it difficult to clarify its multiplicity.

For all structures, the differentiation between H9α and H9β was carried out based on *J* values calculated using the GMMX and PCmodel computer programs.[16,17] Table 1 shows an example of this assignment made for lychnofolide (**1**).

Comparison between experimental and calculated *J* values leaves no doubt regarding the assignment of H9α and H9β. This comparison was undertaken for all other structures (**2** to **4**) leading to the differentiation between H9α and H9β in all cases.

The same care and detail dedicated to structures **1** and **2** were also applied to the ^1H NMR data study of substances **3** and **4**, resulting in the dataset shown in Tables 4 and 5, respectively. Results from COSY spectra corroborated the assignment.

The absence of signal overlapping in all ^1H NMR spectra also allowed easy assignment of the hydrogenated carbons, by means of HMQC experiment results. However, greater caution was needed to

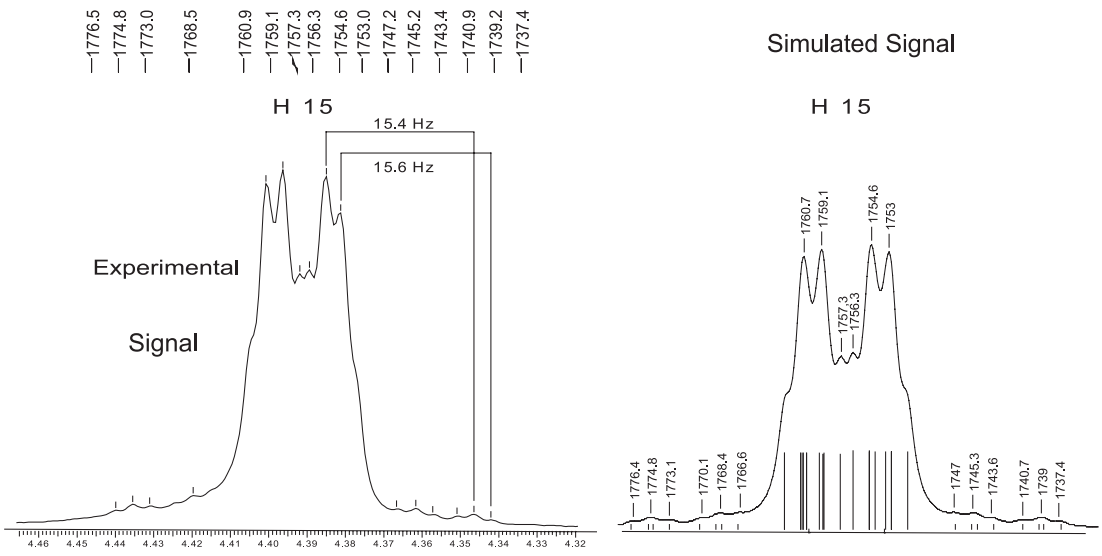


Figure 3. *Experimental and simulated signals for H15 from* ***2***

Table 2. ^{1}H and ^{13}C NMR data for lychnofolide (**1**). 500MHz, $CDCl_3$

C	δ C (ppm)	H	δ H (ppm)	mult.	Coupling constants *J* (Hz)
1	204.9 (C)	---	---	---	---
2	104.7 (CH)	2	5.72 (1H)	*s*	---
3	186.9 (C)	---	---	---	---
4	130.3 (C)	---	---	---	---
5	135.1 (CH)	5	6.01 (1H)	*dq*	*J*(5,6)=3.0; *J*(5,15)=1.7
6	81.7 (CH)	6	5.30 (1H)	*dddq*	*J*(6,5)=3.0; *J*(6,7)=4.9; *J*(6,15)=2.3; *J*(6,8)=0.8
7	51.2 (CH)	7	3.71 (1H)	*ddddd*	*J*(7,6)=4.9; *J*(7,8)=2.3; *J*(7,13a)=2.8; *J*(7,13b)=3.0; *J*(7,9β)=0.8
8	73.0 (CH)	8	4.53 (1H)	*dddd*	*J*(8,7)=2.3; *J*(8,9α)=11.8; *J*(8,9β)=2.0; *J*(8,6)=0.8
9	44.0 (CH_2)	9α	2.48 (1H)	*dd*	*J*(9α,8)=11.8; *J*(9α,9β)=13.9
		9β	2.30 (1H)	*ddd*	*J*(9β,8)=2.0; *J*(9β,9α)=13.9; *J*(9β,7)=0.8
10	89.7 (C)	---	---	---	---
11	133.7 (C)	---	---	---	---
12	168.9 (C)	---	---	---	---
13	124.3 (CH_2)	13a	5.44 (1H)	*dd*	*J*(13a,7)=2.8; *J*(13a,13b)=0.8
		13b	6.22 (1H)	*dd*	*J*(13b,7)=3.0; *J*(13b,13a)=0.8
14	20.7 (CH_3)	14	1.53 (3H)	*s*	---
15	20.4 (CH_3)	15	2.08 (3H)	*dd*	*J*(15,5)=1.7; *J*(15,6)=2.3
1'	167.1 (C)	---	---	---	---
2'	126.4 (C)	---	---	---	---
3'	140.8 (CH)	3'	6.08 (1H)	*qq*	*J*(3',4')=7.3; *J*(3',5')=1.5
4'	15.7 (CH_3)	4'	1.89 (3H)	*dq*	*J*(4',3')=7.3; *J*(4',5')=1.5
5'	20.0 (CH_3)	5'	1.78 (3H)	*quint*	*J*(5',4')=*J*(5',3')=1.5

unequivocally assign the quaternary carbons. In these cases, several ambiguities cited in the literature were clarified by the use of HMBC experiment data. As examples we can highlight the doubt among C12 and C1' assignment and also over C11, C4 and C2' assignment.[1] As can be observed, 2D NMR data in Tables 1S to 4S, supplementary material, can effectively eliminate any assignment mistake.

Relative stereochemistry of all molecules was confirmed by NOEDIFF experiments, as depicted in Figure 1. The most relevant results from these experiments are the observation of nOe between H14 and H9α; between H6 and H8; between H7 and H9α and between H8 and H9β. These results are shown in Figure 4.

CONCLUSION

All sesquiterpene lactones investigated in this study had their unequivocal and complete ^{1}H and ^{13}C NMR data assignments undertaken. Detailed assignment of all hydrogen and carbon chemical shifts was provided with every multiplicity clarified and all hydrogen homonuclear coupling constants measured. The NOEDIFF experiments confirmed all relative stereochemistry for each structure and some previously ambiguous carbon assignments were also clarified.

Table 3. ^{1}H and ^{13}C NMR data for centratherin (**2**). 500MHz, $CDCl_3$

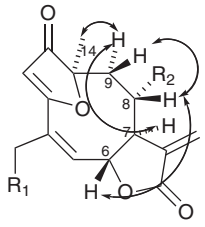
C	δ C (ppm)	H	δ H (ppm)	mult.	Coupling constants *J* (Hz)
1	204.8 (C)	---	---	---	---
2	106.7 (CH)	2	5.82 (1H)	*s*	---
3	184.5 (C)	---	---	---	---
4	134.5 (C)	---	---	---	---
5	135.3 (CH)	5	6.28 (1H)	*dt*	*J*(5,6)=3.1; *J*(5,15)=1.5
6	81.8 (CH)	6	5.37 (1H)	*dddt*	*J*(6,5)=3.1; *J*(6,7)=5.0; *J*(6,15)=2.0; *J*(6,8)=0.9
7	51.0 (CH)	7	3.78 (1H)	*ddddd*	*J*(7,6)=5.0; *J*(7,8)=2.2; *J*(7,13a)=2.6; *J*(7,13b)=3.0; *J*(7,9β)=0.8
8	73.0 (CH)	8	4.53 (1H)	*dddd*	*J*(8,7)=2.2; *J*(8,9α)=11.9; *J*(8,9β)=1.8; *J*(8,6)=0.9
9	44.2 (CH_2)	9α	2.50 (1H)	*dd*	*J*(9α,8)=11.9; *J*(9α,9β)=13.8
		9β	2.32 (1H)	*ddd*	*J*(9β,8)=1.8; *J*(9β,9α)=13.8; *J*(9β,7)=0.8
10	89.9 (C)	---	---	---	---
11	133.5 (C)	---	---	---	---
12	169.0 (C)	---	---	---	---
13	124.6 (CH_2)	13a	5.46 (1H)	*dd*	*J*(13a,7)=2.6; *J*(13a,13b)=0.5
		13b	6.23 (1H)	*dd*	*J*(13b,7)=3.0; *J*(13b,13a)=0.5
14	20.8 (CH_3)	14	1.54 (3H)	*s*	---
15	63.2 (CH_2)	15a	4.38 (1H)	*dddd*	*J*(15a,15b)=14.0; *J*(15a, OH)=6.3; *J*(15a,6)=2.0; *J*(15a,5)=1.5
		15b	4.40 (1H)	*dddd*	*J*(15b,15a)=14.0; *J*(15b, OH)=6.1; *J*(15b,6)=2.0; *J*(15b,5)=1.5
OH	---		2.35 (1H)	*dd*	*J*(OH, 15a)=6.3; *J*(OH, 15b)=6.1
1’	167.3 (C)	---	---	---	---
2’	126.4 (C)	---	---	---	---
3’	140.9 (CH)	3’	6.09 (1H)	*qq*	*J*(3’,4’)=7.3; *J*(3’,5’)=1.5
4’	15.7 (CH_3)	4’	1.89 (3H)	*dq*	*J*(4’,3’)=7.3; *J*(4’,5’)=1.5
5’	20.0 (CH_3)	5’	1.78 (3H)	*quint*	*J*(5’,4’)=*J*(5’,3’)=1.5

Table 4. ^{1}H and ^{13}C NMR data for goyazensolide (**3**). 500MHz, $CDCl_3$

C	δ C (ppm)	H	δ H (ppm)	mult.	Coupling constants *J* (Hz)
1	204.7 (C)	---	---	---	---
2	106.7 (CH)	2	5.83 (1H)	*s*	---
3	184.4 (C)	---	---	---	---
4	134.5 (C)	---	---	---	---
5	135.3 (CH)	5	6.28 (1H)	*dt*	*J*(5,6)=2.9; *J*(5,15)=1.5
6	81.6 (CH)	6	5.34 (1H)	*dddt*	*J*(6,5)=2.9; *J*(6,7)=4.8; *J*(6,15)=2.1; *J*(6,8)=0.6
7	50.9 (CH)	7	3.80 (1H)	*dddt*	*J*(7,6)=4.8; *J*(7,8)=2.6; *J*(7,13a)=3.1; *J*(7,13b)=2.6; *J*(7,9β)=0.8
8	73.3 (CH)	8	4.54 (1H)	*dddd*	*J*(8,7)=2.6; *J*(8,9α)=11.7; *J*(8,9β)=2.1; *J*(8,6)=0.6
9	43.9 (CH_2)	9α	2.51 (1H)	*dd*	*J*(9α,8)=11.7; *J*(9α,9β)=13.8
		9β	2.32 (1H)	*ddd*	*J*(9β,8)=2.1; *J*(9β,9α)=13.8; *J*(9β,7)=0.8
10	89.8 (C)	---	---	---	---
11	133.2 (C)	---	---	---	---
12	168.8 (C)	---	---	---	---
13	124.7 (CH_2)	13a	6.21 (1H)	*dd*	*J*(13a,7)=3.1; *J*(13a,13b)=0.7
		13b	5.48 (1H)	*dd*	*J*(13b,7)=2.6; *J*(13b,13a)=0.7
14	20.7 (CH_3)	14	1.54 (3H)	*s*	---
15	63.2 (CH_2)	15a	4.36 (1H)	*ddd*	*J*(15a,6)=2.1 ; *J*(15a,5)=1.5 ; *J*(15a,15b)=14.5
		15b	4.40 (1H)	*ddd*	*J*(15b,6)=2.1 ; *J*(15b,5)=1.5 ; *J*(15b,15b)=14.5
1’	166.9 (C)	---	---	---	---
2’	135.4 (C)	---	---	---	---
3’	126.6 (CH_2)	3’a	6.02 (1H)	*dq*	*J*(3’a,4’)=0.8; *J*(3’a,3’b)=1.5
		3’b	5.56 (1H)	*dq*	*J*(3’b,4’)=1.2; *J*(3’b,3’a)=1.5
4’	17.9 (CH_3)	4’	1.84 (3H)	*dd*	*J*(4’,3’a)=0.8; *J*(4’,3’b)=1.2

Table 5. ^{1}H and ^{13}C NMR data for goyazensolide acetate (**4**). 500MHz, $CDCl_3$

C	δ C (ppm)	H	δ H (ppm)	mult.	Coupling constants *J* (Hz)
1	204.8 (C)	---	---	---	---
2	107.2 (CH)	2	5,73 (1H)	*s*	---
3	183.7 (C)	---	---	---	---
4	133.5 (C)	---	---	---	---
5	138.9 (CH)	5	6,25 (1H)	*dt*	*J*(5,6)=2.9; *J*(5,15)=1.7
6	81.6 (CH)	6	5,26 (1H)	*dddt*	*J*(6,5)=2.9; *J*(6,7)=4.8; *J*(6,8)=0.6; *J*(6,15a)=2.4; *J*(6,15b)=2.4
7	51.2 (CH)	7	3,71 (1H)	*dddt*	*J*(7,6)=4.8; *J*(7,8)=2.6; *J*(7,13a)=3.1; *J*(7,13b)=2.6; *J*(7,9β)=0.8
8	73.6 (CH)	8	4,46 (1H)	*dddd*	*J*(8,6)=0.6; *J*(8,7)=2.6; *J*(8,9α)=11.6; *J*(8,9β)=1.4
9	44.27 (CH_2)	9α	2,43 (1H)	*dd*	*J*(9α,8)=11.6; *J*(9α,9β)=13.4
		9β	2,25 (1H)	*ddd*	*J*(9β,8)=1.4; *J*(9β,9α)=13.4; *J*(9β, 7)=0.8
10	90.3 (C)	---		---	---
11	130.2 (C)	---		---	---
12	168.9 (C)	---		---	---
13	125.1 (CH_2)	13a	6,16 (1H)	*dd*	*J*(13a,7)=3.1; *J*(13a, 13b)=0.6
		13b	5,40 (1H)	*dd*	*J*(13a,7)=2.6; *J*(13b, 13a)=0.6
14	21.0 (CH_3)	14	1,47 (3H)	*s*	---
15	63.8 (CH_2)	15a	4,74 (1H)	*ddd*	*J*(15a,15b)=13.4; *J*(15a,5)=1.7; *J*(15a,6)=2.4
		15b	4,71 (1H)	*ddd*	*J*(15b,15a)=13.4; *J*(15b,5)=1.7; *J*(15b,6)=2.4
1'	166.8 (C)	---		---	---
2'	135.3 (C)	---		---	---
3'	17.8 (CH_2)	3'a	5,94 (1H)	*dq*	*J*(3'a,3'b)=1.5; *J*(3'a,4')=0.8
		3'b	5,48 (1H)	*quint*	*J*(3'b,3'a)= *J*(3'b,4')=1.5
4'	126.5 (CH_3)	4'	1,76 (3H)	*dd*	*J*(4',3'a)=0.8; *J*(4',3'b)=1.5
1''	168.4 (C)	---		---	---
2''	21.2 (CH_3)	17	2,04 (3H)	*s*	---



R_1 = H for **1**; R_1 = OH for **2** and **3**; R_1 = $OCOCH_3$ for **4**
R_2 = angelate for **1** and **2**; R_2 = metacrilate for 3 and **4**

Figure 4. *Major nOe observed in all sesquiterpene lactones (**1** to **4**)*

SUPPLEMENTARY MATERIAL

In supplementary material, available at http://quimicanova.sbq.org.br, in pdf file, with free access, four tables (1S – 4S) are presented with all 2D NMR correlation data for substances **1** to **4**. Those correlations were obtained from COSY, HMQC and HMBC experiments.

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