SAÚDE-GUIMARÃES, DÊNIA A.; RASLAN, DÉLIO S.; CHIARI, EGLER; DE OLIVEIRA, ALAÍDE B.
Complete assignments of NMR data and assessment of trypanocidal activity of new eremantholide C derivatives
Anais da Academia Brasileira de Ciências, vol. 86, núm. 4, diciembre, 2014, pp. 1563-1571
Academia Brasileira de Ciências
Rio de Janeiro, Brasil

Anais da Academia Brasileira de Ciências,
ISSN (Printed Version): 0001-3765
aabc@abc.org.br
Academia Brasileira de Ciências
Brasil

Anais da Academia Brasileira de Ciências (2014) 86(4): 1563-1571
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201420140167
www.scielo.br/aabc

Complete assignments of NMR data and assessment of trypanocidal activity of new eremantholide C derivatives

DÊNIA A. SAÚDE-GUIMARÃES[1], DÉLIO S. RASLAN[2], EGLER CHIARI[3] and ALAÍDE B. DE OLIVEIRA[4]

[1]LAPLAMED, DEFAR, Escola de Farmácia, Universidade Federal de Ouro Preto, Campus Morro do Cruzeiro, s/n, Bauxita, 35400-000 Ouro Preto, MG, Brasil
[2]Departamento de Química, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, 31207-901 Belo Horizonte, MG, Brasil
[3]Departamento de Parasitologia, ICB, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, 31207-901 Belo Horizonte, MG, Brasil
[4]Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, 31270-901 Belo Horizonte, MG, Brasil

Manuscript received on April 4, 2014; accepted for publication on July 8, 2014

ABSTRACT

Chemical transformations of eremantholide C (**1**), a sesquiterpene lactone that was isolated from *Lychnophora trichocarpha* Spreng. led to five new derivatives: 1',2'- epoxyeremantholide C (**2**), 5-n-propylamine-4,5-dihydro-1',2'-epoxyeremantholide C (**3**), 5-n-propylammonium-4,5-dihydro-1',2'-epoxyeremantholide C chloride (**4**), 5-n-propylammonium-4,5-dihydroeremantolide C chloride (**5**) and 16-*O*-ethyleremantholide C (**6**). The structures of all these derivatives were assigned on the basis of IR, MS, ^{1}H and ^{13}C NMR data by 1D and 2D techniques. Eremantholide C and the derivatives **2**, **4** and **5** were evaluated against trypomastigotes Y and CL strains of *Trypanosoma cruzi*. Eremantholide C completely inhibited the growth of both the parasites strains while all derivatives were partially active against the CL strain and inactive against the Y strain.

Key words: Eremantholide C derivatives, *Lychnophora trichocarpha*, NMR, sesquiterpene lactones, trypanocidal activity.

INTRODUCTION

Sesquiterpene lactones are chemical markers of certain plant families such as *Asteraceae*. The large number of biological activities experimentally described up to now, raised great interest on this group of substances. The sesquiterpene lactones of the furanheliangolide type are biogenetically derived from heliangolides and are often found in species of the genus *Lychnophora*, that is native from Brazil (Bohlmann and Jakupovic 1990). In previous studies, eremantholide C and its oxidized derivatives showed activity against *Trypanosoma cruzi* (Oliveira et al. 1996, Saúde-Guimarães et al. 2007). Other biological/pharmacological activities were reported for eremantholide C, such as antibacterial, anti-hyperuricemic, anti-gouty arthritis, anti-inflammatory and antitumor (Barrero et al. 2000, Saúde et al. 2002, de Souza et al. 2012,

Correspondence to: Dênia Antunes Saúde-Guimarães
E-mails: saude@ef.ufop.br / saudeguima@gmail.com

Ferrari et al. 2013, Saúde-Guimarães et al. 2014). Aiming to obtain new bioactive derivatives of eremantholide C (Figure 1), this sesquiterpene lactone was submitted to chemical modifications.

MATERIALS AND METHODS

General Procedures

Mass spectra were obtained with VG Autospec (electron impact) and HP 5988A (chemical ionization with methane) spectrometers by direct injection (ionization chamber at 200 °C). IR spectra were taken at Galaxy 3000-FTIR spectrophotometer (Mattson Instruments). NMR spectra were taken at Bruker *Avance* DPX (4.7T), DRX (9.4T), equipped with a 5mm dual probe, at 300 K, with TMS as internal reference. One-dimensional ^{1}H and ^{13}C NMR spectra were acquired under standard conditions, with 90° pulse widths of 8.00 μs and 8.50 μs for ^{1}H and ^{13}C, respectively. ^{1}H NMR spectra were obtained using a sweep width of 3 kHz over 32k data points. ^{13}C NMR spectra were obtained using a sweep width of 31 kHz. DEPT, $^{1}H,^{1}H$ COSY and $^{1}H,^{13}C$ HETCOR techniques were performed using standard pulse sequences supplied by the spectrometer manufacturer.

Synthesis of Eremantholide C derivatives 2 to 6

Eremantholide C (**1**) was obtained from aerial parts of *L. trichocarpha* Spreng. that were collected in Minas Gerais, Brazil, as previously described (Saúde et al. 1998, Ferrari et al. 2013). Its structural characterization is described elsewhere (Le Quesne et al. 1978, Saúde et al. 1998, Saúde-Guimarães et al. 2007).

1',2'-Epoxyeremantholide C (**2**) was prepared by reaction of **1** (2.9 mmol) with *m*-chloroperbenzoic acid (5.3 mmol) in 50 mL of anhydrous $CHCl_3$. The mixture was stirred at room temperature for 2 h. The reaction mixture was worked out according to Carda et al. 1986. The resulting residue (1.14 g) was purified by column chromatography (silica gel, hexane: EtOAc 1:1), yielding 970 mg of **2** (93% yield, m.p. 213-217 °C).

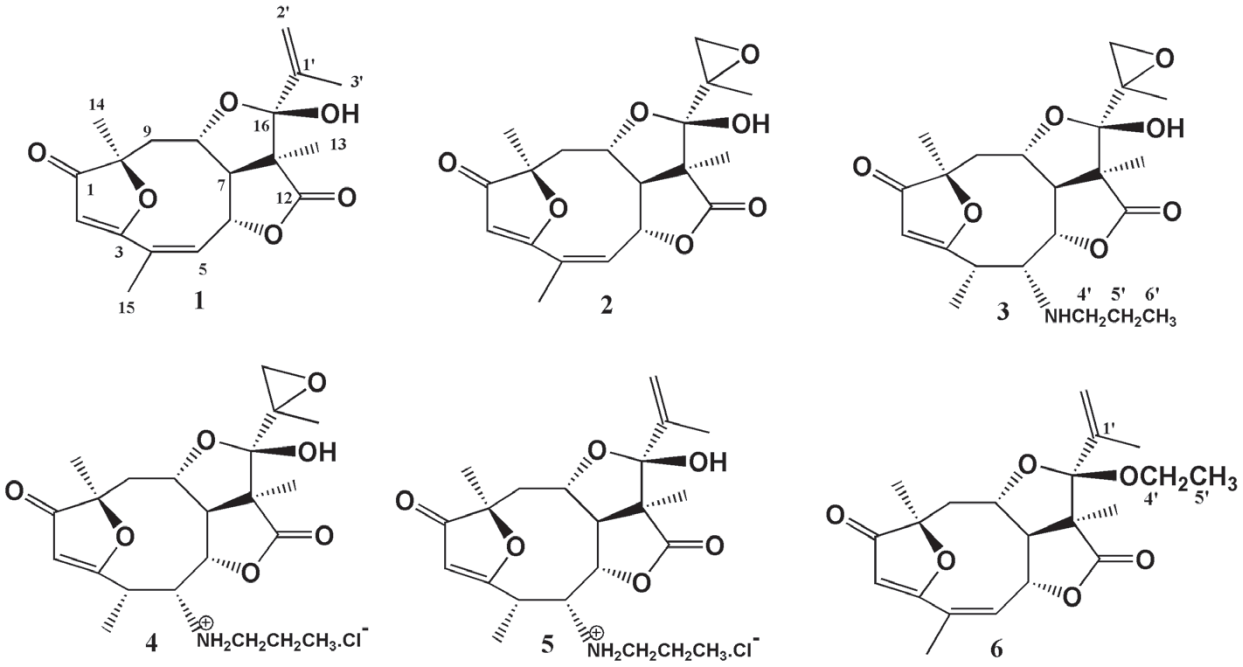

Figure 1 - Eremantholide C and derivatives **2-6.**

1',2'-Epoxyeremantholide C (2): 6,9-Epoxy-2*H*-1,4-dioxacyclodeca[c,d]pentalene-2,7(4a*H*)-dioxane,2a,3,5,6,11a,11b-hexahydro-3-hydroxy-2a,6,10-trimethyl-3-(1',2'-epoxy)-2a*R**,3*S**,4a*R**,6*S**,10*Z*,11a*S**,11b*S**). White solid, m.p. 213-217°C. IR (KBr) ν_{max} (cm^{-1}): 3400 (OH), 1770 (C=O, γ-lactone), 1700 (ketone C=O), 1650 (C=C), 1590 (C=COR, furanone), 1450, 1350, 1300, 1275, 1150, 1100, 1050, 1000, 900. MS (EI), *m/z* (rel. int.): 362 [M^+, $C_{19}H_{22}O_7$] (20), 344 (M - H_2O, 17), 234 (19), 205 (19), 189 (20), 177 (29), 165 (32), 149 (25), 138 (55), 122 (53), 95 (**100**), 69 (58), 57 (22).

5-n- Propylamine -4,5-dihydro-1',2'-epoxyeremantholide C **(3)** was obtained by reaction of **2** (0.55 mmol) with n-propylamine (5.94 mmol), at -18 °C for 16 h (Kirk 1973). After this period, the excess of the amine was removed by evaporation under reduced pressure, at room temperature. The residue obtained was purified by PTLC on silica gel (0.5 mm thickness, eluent - hexane: EtOAc 1:1) resulting in 82 mg (35% yield) of **3** as a white solid. This compound was dissolved in dry THF and treated with gaseous HCl leading to the formation of **4** as a white hydrosoluble solid (quantitative yield).

5-n-Propylamine-4,5-dihydro-1',2'-epoxyeremantholide C (3): White solid, m.p. 183-189 ^{0}C. IR (KBr) ν_{max} (cm^{-1}): 3400 (OH), 1770 (C=O, γ-lactone), 1700 (C=O, ketone), 1590 (C=COR, furanone), 1450, 1380, 1300, 1200, 1150, 1100, 1050, 1000, 900, 850. MS (EI), *m/z* (rel. int.): 421 (M^+, $C_{22}H_{31}O_7N$, **99**), 403 (M - H_2O, 28), 238 (25), 195 (64), 168 (31), 151 (25), 138 (33), 125 (**100**), 99 (23), 72 (35).

5-n-Propylammonium-4,5-dihydro-1',2'-epoxyeremantholide C chloride (4): white solid, m.p. 176-179 ^{0}C. MS (EI), *m/z* (rel. int.): 457 (M^+, $C_{22}H_{32}O_7NCl$, 72), 439 (M - H_2O, 11), 390 (18), 364 (31), 336 (21), 318 (59), 195 (79), 168 (44), 152 (19), 138 (45), 125 (**100**), 99 (45), 81 (18), 72 (46).

5-n-Propylammonium-4,5-dihydroeremantolide C chloride (**5**) was obtained from the reaction of **1** (0.58 mmol) with n-propylamine (5.94 mmol), at -18 °C for 10 h (Kirk 1973). After this period, the excess of amine was removed by evaporation under reduced pressure, at room temperature. The residue obtained was purified by PTLC on silica gel (0.5 mm thickness, eluent - hexane: EtOAc 1:1) affording 120 mg of a yellowish pasty material. Silica TLC of this material showed three spots when revealed by iodine. After development with ninhydrin, the TLC showed only a rosy spot, characteristic of amines. This material was then dissolved in dry THF and treated with gaseous HCl to give the hydrochloride **5** as a white hydrosoluble solid **(**70 mg).

5-n-Propylammonium-4,5-dihydroeremantolide C chloride (5): White solid, m.p. 195-197 ^{0}C. MS (EI), *m/z* (rel. int.): 405 (60), 387 (M - H_2O, 5), 195 (40), 168 (32), 135 (21), 125 (**100**), 69 (35).

16-*O*-Ethyleremantholide C (**6**) was obtained, according to the methodology described by Partwardhan et al. 1974, by reacting **1** (0.28 mmol) with 0.08 mL of triethyl orthoformate and 25 mg of Amberlyst resin 15. The reaction mixture was stirred at room temperature for 3 days. Then it was neutralized with aqueous K_2CO_3 solution and filtered. The product was extracted with diethyl ether. The organic layer was washed with water, filtered on anhydrous sodium sulfate and concentrated under reduced pressure, yielding 72 mg of ether **6** as a white solid (66% yield).

16-*O*-Ethyleremantholide C (6): White solid. IR (KBr) ν_{max} (cm^{-1}): 1770 (C=O, γ-lactone), 1700 (C=O, ketone), 1660 (C=C), 1590 (C=COR, furanone), 1450, 1380, 1300, 1280, 1260, 1210, 1200, 1150, 1140, 1100, 1060, 1000, 870, 750. MS (EI), *m/z* (rel. int.): 375 $(M+1)^+$, $C_{21}H_{26}O_6$, 3), 329 (54), 285 (17), 199 (9), 165 (49), 95 (85), 69 (**100**), 67 (18), 45 (23), 43 (39).

In vitro Assays with *Trypanosoma cruzi* Trypomastigotes

Albino mice infected with the Y or CL strains of *T. cruzi* were used to provide trypomastigotes.

Samples of eremantholide C and of the derivatives **2**, **4** and **6** were dissolved or suspended in dimethyl sulfoxide (DMSO) (0.2 mL) and plus Krebs-Ringer-glucose (2.0 mL) and mixed with an equal volume of parasitized whole blood diluted in bovine calf serum. A parasite density of 2 x 10^5 trypomastigotes/0.1 mL was calculated for each flat-bottomed test tube **(**4 mL, 56 x 13 mm); control tubes without the test extracts were included. After incubation at 4 °C for 24 h the suspensions were examined microscopically. Only those samples that killed 100% of the parasites were considered active. Samples that inhibited 50% of parasite growth compared to control were considered partially active.

RESULTS AND DISCUSSION

Data of the ^{1}H and ^{13}C NMR spectra of derivatives **2-6** are given at on Tables I and II, respectively. The chemical shifts were assigned by consideration of known substituent effects of the groups concerned and with the aid of both ^{1}H,^{1}H COSY and ^{1}H,^{13}C HETCOR contour maps. The stereochemistry of the carbons were determined based on coupling constants and ^{1}H,^{1}H NOESY contour maps.

From the reaction of **1** with *m*-chloroperbenzoic acid, the main product **2** was obtained and it was characterized by ^{1}H NMR data. The presence of an epoxy group in **2** was highlighted in the ^{1}H NMR spectrum by the two doublets at δ3.11 (J = 5.5 Hz) and δ2.70 (J = 5.5 Hz), attributed to H-2'a and H-2'b, which appear in the ^{1}H NMR spectrum of **1** at δ5.31 (br s) and δ5.07 (t, J = 1.6 Hz), respectively.

The signals at δ130.00 and δ115.80, attributed to the olefinic carbons C-1' and C-2', respectively, in the ^{13}C NMR spectrum of **1,** are replaced in the spectrum of **2** by the signals at δ59.50 and δ58.47, typical chemical shifts of oxygenated carbons with hybridization sp^3. In addition, the signal attributed to C-3 in the spectrum of **1 (**δ19.00) is shifted to δ11.44 in the spectrum of **2**, confirming the epoxidation of the Δ^1 double bond of **1**.

The mass spectrum of **2** showed the molecular ion peak at m/z 362, which represents an increment of 16 mass units to the molar mass of **1**, corresponding to the addition of an oxygen atom. This mass is consistent with the molecular formula $C_{19}H_{22}O_7$.

Data of IR, MS, ^{1}H NMR and ^{13}C NMR of **2**, are in agreement with the new derivative 1',2'-epoxyeremantholide C.

Epoxide **2** was reacted with methylamine, cyclohexylamine, diethylamine and n-propylamine. Reactions with the first three amines led to mixtures of many products, in such a way that no derivative with suitable purity was obtained. The reaction of **2** with n- propylamine afforded a less complex mixture of products which, after separation by column chromatography on silica gel, led to the amino product **3** with a 35% yield. The amine **3** was transformed into its hydrochloride salt by reaction with gaseous HCl in dry THF, leading to the hydrosoluble compound **4**.

The multiplets in δ5.02-4.98 and δ6.03-6.00 attributed, respectively, to H-6 and H-5, in the ^{1}H NMR spectrum of **2** appear in the spectrum of **3** at δ4.26 (d, J = 6.5 Hz) and at δ2.99 (brs), respectively. The quartet at δ2.89 (J = 7.2 Hz), that appears in the ^{1}H NMR spectrum of **2**, was assigned to H-4. A correlation of this signal with the doublets at δ1.37 (J = 7.2 Hz), assigned to H-15, is indicated by the values of the coupling constants and by the COSY ^{1}H-^{1}H contour map. The signals at δ134.27 and δ130.24 which are attributed to the olefinic carbons C-5 and C-4, respectively, as well as the signal attributed to C-15 (δ20.39) in the ^{13}C NMR spectrum of **2**, are shifted in the ^{13}C NMR spectrum of **3** to δ65.24, δ38.20 and δ16.70, respectively, allowing the proposition that the amine group was linked to the C-5 of compound **2**.

The double triplets at δ2.80 (J = 7.0 and 11.2 Hz) and δ2.53 (6.9 and 11.2 Hz) assigned to H-4'a and b, respectively, the multiplet at δ1.50 assigned to H-5', and the triplet at δ0.93 (J = 7.3 Hz) assigned to the atoms H-6'of the methyl group,

TABLE I
^{1}H NMR data for compounds 1, 2, 3, 4, 5 and 6, δ, J (Hz).

H	1*, 1	2**, 1	3**, 1	4**, 2	5**, 2	6**, 1
2	5.63 s	5.62 s	5.56 s	5.99 s	5.93 s	5.62 s
4	-	-	2.89 q $J_{4,15}$ = 7.2	3.56 q $J_{4,15}$ = 7.2	3.45 q $J_{4,15}$ = 7.2	-
5	6.04-6.03 m	6.03-6.00 m	2.99 brs	3.97 brs	3.88 brs	6.03-6.01 m
6	5.02-4.98 m	5.02-4.97 m	4.26 d $J_{6,7}$ = 6.5	4.74 a	4.73 d $J_{6,7}$ = 7.9	4.90-4.86 m
7	2.82 dd $J_{6,7}$ = 7.1; $J_{7,8}$ = 4.2	2.87 dd $J_{6,7}$ = 7.3; $J_{7,8}$ = 4.2	2.95 dd $J_{6,7}$ = 6.5; $J_{7,8}$ = 4.9	3.09 dd $J_{6,7}$ = 7.8; $J_{7,8}$ = 4.2	3.04 dd $J_{6,7}$ = 7.8; $J_{7,8}$ = 4.3	2.84 dd $J_{6,7}$ = 7.2; $J_{7,8}$ = 4.4
8	4.09 ddd $J_{7,8}$ = 4.2; $J_{8,9a}$ = 2.5; $J_{8,9b}$ = 12.0	4.07 ddd $J_{7,8}$ = 4.2; $J_{8,9a}$ = 2.6; $J_{8,9b}$ = 11.9	3.91 ddd $J_{7,8}$ = 4.9; $J_{8,9a}$ = 2.3; $J_{8,9b}$ = 11.9	3.92 m	3.96 ddd $J_{7,8}$ = 4.3; $J_{8,9a}$ = 2.8; $J_{8,9b}$ = 113	3.85 ddd $J_{7,8}$ = 4.4; $J_{8,9a}$ = 2.4; $J_{8,9b}$ = 12.0
9a	2.48 dd $J_{8,9a}$ = 2.5; $J_{9a,9b}$ = 13.5	2.39 dd $J_{8,9a}$ = 2.6; $J_{9a,9b}$ = 13.6	2.39 dd $J_{8,9a}$ = 2.3; $J_{9a,9b}$ = 13.5	2.32 dd $J_{8,9a}$ = 2.3; $J_{9a,9b}$ = 13.6	2.27 dd $J_{8,9a}$ = 2.8; $J_{9a,9b}$ = 13.8	2.41 dd $J_{8,9a}$ = 2.4; $J_{9a,9b}$ = 13.6
9b	2.00 dd $J_{8,9b}$ = 12.0; $J_{9a,9b}$ = 13.5	1.94 dd $J_{8,9b}$ = 11.9; $J_{9a,9b}$ = 13.6	1.90 dd $J_{8,9b}$ = 11.9; $J_{9a,9b}$ = 13.5	2.39 $J_{8,9b}$ = 11.5; $J_{9a,9b}$ = 13.6	2.34 dd $J_{8,9b}$ = 11.3; $J_{9a,9b}$ = 13.8	2.06 dd $J_{8,9b}$ = 12.0; $J_{9a,9b}$ = 13.6
13	1.18 s	1.33 s	1.38 s	1.39 s	1.21 s	1.16 s
14	1.45 s	1.48 s	1.43 s	1.50 s	1.45 s	1.50 s
15	2.05 t $J_{5.15}$ =$_{6,15}$ = 1.9	2.05 t $J_{5,15}$ =$_{6,15}$ = 2.0	1.37 d $J_{4,15}$ = 7.2	1.52 d $J_{4,15}$ = 7.2	1.48 d $J_{4,15}$ = 7.2	2.06 dd $J_{5,15}$ = 2.0; $J_{6,15}$ = 1.6
2'a	5.31 brs	3.11 d $J_{2'a,2'b}$ = 5.5	3.12 d $J_{2'a,2'b}$ = 5.5	4.04 d $J_{2'a,2'b}$ = 11.9	5.22 brs	5.28 d $J_{2'a,2'b}$ = 1.2
2'b	5.07 t $J_{2'a,2'b}$ = 1.6	2.70 d $J_{2'a,2'b}$ = 5.5	2.71 d $J_{2'a,2'b}$ = 5.5	3.75 d $J_{2'a,2'b}$ = 11.9	5.13 t $J_{2'a,2'b}$ = 1.4	5.13 brs
3'	1.91 s	1.55 s	1.57 s	1.50 s	1.77 s	1.79 s
4'a	-	-	2.8 dt $J_{4'a,4'b}$ = 11.2; $J_{4'a,5'}$ = 7.0	3.28 td $J_{4'a,4'b}$ = 11.2; $J_{4'a,5'}$ = 5.8;	3.18 dt $J_{4'a,4'b}$ = 10.5; $J_{4'a,5'}$ = 5.7	3.36 dq $J_{4'a,4'b}$ = 9.2; $J_{4'a,5'}$ =7.2
4'b	-	-	2.53 dt $J_{4'a,4'b}$ = 11.2; $J_{4'b,5'}$ = 6.9;	3.17 td $J_{4'a,4'b}$ = 11.2; $J_{4'b,5'}$ =5.8	3.09 dt $J_{4'a,4'b}$ = 10.5; $J_{4'b,5'}$ =5.7	3.20 m
5'	-	-	1.50 m	1.80 m	1.75 m	1.07 t $J_{4'a,5'}$ =$_{,4'b,5'}$ = 7.2
6'	-	-	0.93 t $J_{5',6'}$ = 7.3	0.99 t $J_{5',6'}$ = 7.4	0.94 t $J_{5',6'}$ = 7.3	-
OH	3.79 s	3.69 s				

x – Superimposed signal with D_2O. * - 300 MHz; ** - 400 MHz, 1 - $CDCl_3$, 2 - D_2O.

indicated the presence of a propylamine group at **3**. This group was confirmed by the ^{13}C NMR signals at δ50.51, δ17.52 and δ11.65, attributed to carbons 4', 5' and 6', respectively.

TABLE II
^{13}C NMR data (δ) for 1, 2, 3, 4, 5 and 6.

C	1 *,1	2**,1	3**,1	4**,2	5**,2	6**,1
C-1	205.89	205.23	204.53	209.06	209.34	205.24
C-2	104.54	104.50	104.03	105.93	106.08	104.45
C-3	187.27	186.64	192.93	192.39	192.53	186.91
C-4	130.00	130.15	38.14	33.44	33.68	139.10
C-5	134.77	134.28	57.18	58.65	57.30	134.75
C-6	81.46	81.34	82.42	80.77	81.00	81.75
C-7	62.53	63.06	65.24	63.79	63.99	63.10
C-8	78.37	78.38	77.45	76.47	77.05	78.43
C-9	43.46	43.25	43.84	42.38	42.60	43.89
C-10	90.24	89.95	89.88	91.88	91.99	89.98
C-11	59.88	58.43	58.51	61.65	60.61	60.00
C-12	175.72	174.70	175.09	177.21	178.19	175.76
C-13	21.94	20.64	21.16	21.52	20.83	21.66
C-14	20.48	20.39	20.51	20.16	20.22	20.64
C-15	20.30	20.33	16.70	15.06	15.17	20.49
C-16	106.09	104.57	104.94	107.06	107.27	109.59
C-1'	142.22	59.60	59.71	76.67	141.39	139.28
C-2'	115.80	53.59	53.80	50.06	116.63	117.61
C-3'	19.00	11.44	17.55	20.07	19.19	18.46
C-4'	-	-	50.51	49.11	49.22	57.26
C-5'	-	-	17.52	19.04	18.70	15.29
C-6'	-	-	11.65	10.52	10.62	-

* - 75 MHz; ** - 100 MHz; 1 - $CDCl_3$; 2 - D_2O.

The two doublets at δ2.71 (J = 5.5 Hz) and δ3.12 (J = 5.5 Hz) assigned to H-2'a and b, respectively, and the signals at δ59.71 and δ53.80 on the ^{13}C NMR spectrum, due to carbons 1' and 2', respectively, showed that the epoxy group remained unchanged in the molecule of **3**.

The assignments made to the signals in the ^{1}H NMR spectrum of **3** were confirmed by the values of the coupling constants and by the ^{1}H-^{1}H COSY contour map which showed the following correlations: H-4 and H-15; H-4'a, H-4'b and H-5'; H-5' and H-6'; H-6 and H-7, and H-2'a and b. Chemical shifts for ^{13}C NMR spectrum of **3** were assigned by comparison with the spectrum of **2** and based on the DEPT spectrum.

The mass spectrum of **3** presented the molecular ion peak at m/z 421 u, corresponding to 59 mass units higher than the molecular ion of **2**, and it corresponds to n-propylamine group ($C_3H_7NH_2$). The molar mass of **3** was compatible with the molecular formula $C_{22}H_{31}O_7N$.

Based on data retrieved from IR, MS, ^{1}H and ^{13}C NMR it was concluded that the product obtained was that resulting from the amine addition to carbon-5 of the epoxide **2**, through a Michael's reaction, generating the novel derivative 5-n-propylamine-1',2'-epoxyeremantholide (**3**). This might be compared to the proposed mechanism for the antitumor activity of the eremantholides by reaction with biological nucleophiles (Mc Dougal et al. 1989).

The shifting of the signals due to the hydrogen and carbon atoms 4, 5, 15, 4'a, 4'b, 5' e 6' of compounds **3** and **4** could be observed by comparing their ^{1}H and ^{13}C NMR spectra.

The signals relative to H-4, H-5, H-15, H-4'a and H-4'b, H-5' and H-6' appear in the spectrum of **3**, respectively, at δ2.89 (q, J = 7.2 Hz), δ2.99 (brs), δ1.37 (d, J = 7.2 Hz), δ2.80 (dt, J = 7.0 and 11.2 Hz), δ2.53 (dt, J = 6.9 and 11.2 Hz), δ1.50 (m) and δ0.93 (t, J = 7.3 Hz), while in the spectrum of **4** they are shown at δ3.56 (q, J = 7.2 Hz), δ3.97 (brs), δ1.52 (d J = 7.2 Hz), δ3.28 (td, J = 5.8 and 11.2 Hz), δ3.17 (td, J = 5.8 and 11.2 Hz), δ1.80 (m) and δ0.99 (t, J = 7.4 Hz), respectively.

In the ^{13}C NMR spectrum of **3**, the signals at δ 38.20, δ 57.18, δ 16.70, δ 50.51, δ17.52 and δ11.65 were assigned to C-4, C-5, C-15, C-4', C-5' and C-6', respectively. These signals were observed at δ33.44, δ58.65, δ15.06, δ49.11, δ19.04 and δ10.52, respectively, in the spectrum of **4**.

The signal corresponding to H-6 in the ^{1}H NMR spectra of **4** overlapped that of the solvent (D_2O) at δ4.74. This was confirmed by the ^{1}H-^{1}H COSY contour map of **4,** evidencing correlations between the signal at δ4.74 (brs), and the signals attributed to H-7 (δ3.09, dd, J = 4.2 and 7.8 Hz),

and to H-5 (δ3.97, brs). The ^{1}H-^{1}H COSY also evidenced correlations between the following hydrogen atoms: H-4 and H-15; H-8, H-9a and H-9b; H-9a and H-9b; H-4'a, H-4'b and H-5'; H-5'and H-6'; H-2'a, H-2'b and H-3'.

The relative configuration of the n-propylamine and the C-15-methyl group was defined on the basis of the ^{1}H,^{1}H NOESY (Figure 2), that indicated correlation between the H-5 signal at δ3.97 (brs) , with the signals at δ4.74 and δ3.56 (q, J = 7.2 Hz), attributed to H-6 and H-4, respectively. This correlation showed a *–cis* relationship between the H-4 and H-5 atoms with a β configuration. Consequently, the C-15-methyl group and the n-propylamine group should have an α configuration.

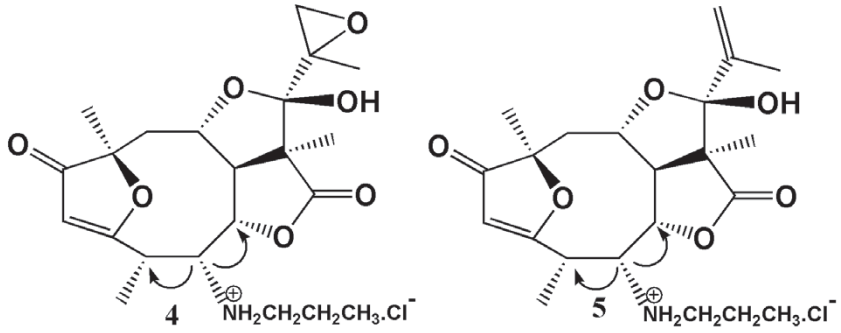

Figure 2 - Arrows represent NOESY correlations.

The mass spectrum of **4** presented a molecular ion peak m/z 457 u, compatible with a molecular formula $C_{22}H_{32}O_7NCl$. Such a molecular mass represents an increment of 36 units compared with the mass of **3**, compatible with the addition of one HCl per molecule of **4**.

Based on data from IR, MS, ^{1}H and ^{13}C NMR, COSY and NOESY it was concluded that compound **4** is 5-n-propylammonium-4,5-dihydro-1',2'-epoxyeremantholide C chloride, a novel compound that is firstly described in this paper.

In order to confirm the positioning of the n-propylamine group at the C-5 of compound **2**, the reaction of **1** with n-propylamine was carried out. Three spots were observed when a silica gel TLC plate of the reaction mixture was sprayed with iodine. Under ninhydrin, only a rosy spot typical of amine, was observed. Then, the mixture was treated with gaseous HCl in dry THF yielding **5** as a white hydrosoluble solid.

In the ^{1}H NMR spectrum of **5**, the signals at δ3.18 (td, J = 5.7 and 10.5 Hz), δ3.09 (td, J = 5.7 and 10.5 Hz), δ1.75 (m) and δ0.94 (t, J = 7.3 Hz) were attributed to H-4'a, H-4'b, H-5'and H-6' of the n-propylammonium group, respectively. These data were further confirmed by the signals at δ49.22 and δ18.70, together with the signal at δ10.62 in the ^{13}C NMR of **5**, which were attributed, respectively, to C-4', C-5' and C-6' of the n- propylammonium group.

The ^{1}H NMR spectrum of **5** presented a quartet at δ3.45 (1H, J = 7.2 Hz) that was attributed to H-4. The signals for H-5 and H-15 in this spectrum appeared as a broad singlet at δ3.88 and a doublet at δ1.48 (J=7.2 Hz), respectively. In the ^{1}H NMR spectrum of **1** these atoms are represented by signals at δ6.04−6.03 (m) and δ2.05 (t, J = 1.9 Hz), respectively. Values of coupling constants for H-4 and H-15 indicate that these hydrogens are in vicinal positions.

The signal attributed to H-6 in the ^{1}H NMR spectra of **5** is probably superimposed to that of H_2O in the solvent (D_2O), which appear at δ4.74. In order to shift the signal of H_2O and observe the one from H-6, the ^{1}H NMR spectra of **5** in D_2O was run at 330 K, when the signal due to the solvent was shifted to δ4.72 and the doublet attributed to H-6 was seen at δ5.07 (J = 7.9 Hz).

The stereochemistry of the n-propylammonium and of the C-15 methyl groups were inferred based on the NOESY technique (Figure 2), which indicated a correlation between the signal at δ3.88 (brs), attributed to H-5, and the signals at δ4.73 (d, J = 7.9 Hz) and δ 3.45 (q, J = 7.2 Hz), corresponding to H-6 and H-4, respectively. These data suggest that H-4 and H-5 have a *cis* relationship and a β configuration. Consequently, the C-15 methyl and the n- propylammonium groups have a α configuration.

The molecular ion peak m/z 441 u, corresponding to the molecular formula $C_{22}H_{32}O_6NCl$, was not observed at the mass spectrum of **5**, but it showed a peak at m/z 405 u that was compatible to the loss of a molecule of HCl from the molecular ion.

Data of IR, MS, ^1H and ^{13}C NMR spectra, and the COSY and NOESY contour maps are in agreement with the new derivative 5-n- propylammonium-4,5-dihydroeremantholide C chloride, a new derivative of eremantholide C.

The infrared spectrum of **6** showed no absorption characteristic of the hydroxyl group. The ^1H NMR spectrum presented signals at δ3.36 (dq, J = 7.2 and 9.2 Hz), δ3.20 (m) and δ1.07 (t, J = 7.2 Hz) corresponding to H-4'a, H-4'b and H-5', respectively, and that indicates the presence of an ethoxyl group. The COSY ^1H,^1H contour map evidenced correlations between H-4'a, H-4'b and H-5' atoms.

The ^{13}C NMR spectrum of **6** shows a signal at δ57.26, characteristic for a sp^3 carbon bonded to oxygen and, therefore, it was attributed to C-4'. The signal at δ15.29 was assigned to the methyl carbon at the 5' position. These data confirmed the presence of an ethoxyl group in the molecule of **6**.

In the mass spectrum of **6** the ion $[M+1]^+$ appeared at m/z 375 u corresponding to an increase of 29 mass units in comparison with **1**, that is compatible with the presence of an ethyl group, and corresponding to the molecular formula $C_{21}H_{26}O_6$.

Assignments of the signals in the ^1H NMR spectrum of **6** were confirmed through the correlations observed at the ^1H, ^1H COSY contour map.

Based on the modifications observed in the IR, MS, ^1H and ^{13}C NMR spectra it was concluded that the compound obtained was the new derivative 16-*O*-ethyleremantholide C (**6**).

Eremantholide C derivatives **2**, **4** and **5** were tested against trypomastigote forms of Y and CL strains of *Trypanosoma cruzi*, the infectious agent of Chagas' disease, in comparison with Violet crystal (active at 125 $\mu g.mL^{-1}$) that was used as reference in the *in vitro* tests. The results are shown on Table III. Eremantholide C and the derivatives **2**, **4** and **5** were evaluated against Y and CL strains of *T. cruzi*. Eremantholide C completely inhibited the growth of both the parasite strains in the concentrations of 3,600 μg/mL (Y strain) and 1,800 μg/mL (CL strain), respectively, while all the derivatives were partially active against the CL strain and inactive against the Y strain, in the concentrations assayed.

TABLE III
Results of *in vitro* tests of compounds 1, 2, 4 and 5 against Y and CL trypomastigote strains of *T. cruzi*. Inhibition of growth (%).

Compound	Y Strain (μg/mL)	CL Strain (μg/mL)
1	3600 (100%)	1800 (100%)
2	3290 (NI)	823 (50%)
4	4155 (NI)	1039 (50%)
5	4009 (NI)	1002 (50%)

NI - Growth was not inhibited.

CONCLUSIONS

The present paper describes the synthesis of five new eremantholide C derivatives (**2-6**) that were spectroscopically characterized and had their activity evaluated *in vitro* against trypomastigotes of Y and CL strains of *T. cruzi.* All the eremantholide C derivatives tested (**2, 4** and **5)** showed 50% growth inhibition of the CL strain.

ACKNOWLEDGMENTS

The authors acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico

(CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and fellowships.

RESUMO

Transformações químicas realizadas em eremantolida C (**1**), uma lactona sesquiterpênica isolada de *Lychnophora trichocarpha* Spreng. originaram cinco novos derivados: 1',2'-epoxieremantolida C (**2**), 5-n-propilamino-4,5-diidro-1',2'-epoxieremantolida C (**3**), cloreto de 5-n-propilamônio-4,5-diidro-1', 2'-epoxieremantolida C (**4**), cloreto de 5-n-propilamônio-4,5-diidroeremantolida C (**5**) e 16-*O*-etileremantolida C (**6**). As estruturas químicas de todos estes derivados foram elucidadas com base nos espectros de IV, EM, RMN de ^{1}H e de ^{13}C e por meio de técnicas 1D e 2D. Eremantolida C e os derivados **2**, **4** e **5** foram avaliados frente a cepas tripomastigotas Y e CL de *Trypanosoma cruzi*. Eremantolida C inibiu completamente o crescimento de ambas as cepas de parasitas, enquanto todos os derivados foram parcialmente ativos contra a cepa CL e inativos contra a cepa Y.

Palavras-chave: Derivados de eremantolida C, *Lychnophora trichocarpha*, RMN, lactonas sesquiterpênicas, atividade anti-*Trypanosoma curuzi*.

REFERENCES

Barrero AF, Oltra JE, Álvarez M, Raslan DS, Saúde DA and Akssira M. 2000. New sources and antifungal activity of sesquiterpene lactones. Fitoterapia 71: 60-64.

Bohlmann F and Jakupovic J. 1990. Progress in the chemistry of the *Vernoniae*. Pl Syst Ecol 4: 3-43.

Carda M, Arnó M and Marco JA. 1986. Total synthesis of Rothin -A and Rothin-B. Tetrahedron 42: 3655-3662.

De Souza MR, De Paula CA, Resende MLP, Grabe-Guimarães A, De Souza Filho JD and Saúde-Guimarães DA. 2012. Pharmacological basis for use of *Lychnophora trichocarpha* in gouty arthritis: anti-hyperuricemic and anti-inflammatory effects of its extract, fraction and constituents. J Ethnopharmacol 142: 845-850.

Ferrari FC, Ferreira LC, Souza MR, Grabe-Guimarães A, Paula CA, Rezende SA and Saúde-Guimarães D A. 2013. Anti-Inflammatory Sesquiterpene Lactones from Spreng. (Brazilian Arnica). PTR Phytother Res 27: 384-389.

Kirk DN. 1973. Selectivity in reactions of epoxides. Chem Ind 3:109-112.

Le Quesne PW, Levery SB, Menachery MD, Brennan TF and Raffauf RF. 1978. Novel Modified Germacranolides and Other Constituents of *Eremanthus elaegnus* Schultz-Bip. (Compositae). J Chem Soc Perkin Trans I 12:1572-1580.

Mc Dougal PG, Oh YI and Van Derveer DJ. 1989. Synthesis of the Furanoheliangolide Ring Skeleton. J Org Chem 54: 91-97.

Oliveira AB, Saúde DA, Perry KSP, Duarte DS, Raslan DS, Boaventura MAD and Chiari E. 1996. Trypanocidal sesquiterpenes from *Lychnophora* species. Phytother Res 10: 292-295.

Patwardhan AS and Dev S. 1974. Amberlyst-15, a superior catalyst for the preparation of enol ethers and acetals. Synthesis 5: 348-349.

Saúde DA, Barrero AF, Oltra JE, Justicia J, Raslan DS and Silva EA. 2002. Atividade Antibacteriana de Furanoeliangolidos. Rev Bras Farmacogn 12: 7-10.

Saúde DA, Raslan DS, De Souza Filho JD and DE Oliveira AB. 1998. Constituents from the aerial parts of *Lychnophora trichocarpha*. Fitoterapia 69: 90-91.

Saúde-Guimarães DA, Perry KSP, Raslan DS, Chiari E, Barrero AF and Oltra JE. 2007. Complete assignments of ^{1}H and ^{13}C NMR data for trypanocidal eremantholide C oxide derivatives. Magn Reson Chem 45: 1084-1087.

Saúde-Guimarães DA, Raslan DS and Oliveira AB. 2014. *In vitro* antitumor activity of sesquiterpene lactones from *Lychnophora trichocarpha*. Rev Bras Plant Med 16: 275-282.