Eclética Química
ISSN: 0100-4670
atadorno@iq.unesp.br
Universidade Estadual Paulista Júlio de Mesquita Filho
Brasil

Alcântara, A. F. C.; Silveira, D.; Chiari, E.; Oliveira, A. B.; Guimarães, J. E.; Raslan, D. S.
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Eclética Química, vol. 30, núm. 3, july-september, 2005, pp. 37-45
Universidade Estadual Paulista Júlio de Mesquita Filho
Araraquara, Brasil

ECLÉTICA Química
www.scielo.br/eq
Volume 30, número 3, 2005

Comparative analysis of the trypanocidal activity and chemical properties of *E*-lychnophoric acid and its derivatives using theoretical calculations

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Abstract: *E*-Lychnophoric acid **1**, its derivative ester **2** and alcohol **3** killed 100% of trypomastigote blood forms of *Trypanosoma cruzi* at the concentrations of 13.86, 5.68, and 6.48 μg/mL, respectively. Conformational distribution calculations (AM1) of **1**, **2** and **3** gave minimum energies for the conformers **a**, **b**, **c,** and **d**, which differ from each other only in the cyclononene ring geometry. Calculations (DFT/BLYP/6-31G*) of geometry optimization and chemical properties were performed for conformers of **1**, **2**, and **3**. The theoretical results were numerically compared to the trypanocidal activity. Calculated values of atomic charge, orbital population, and vibrational frequencies showed that the C-4–C-5 π-endocyclic bond does not affect the trypanocidal activity of the studied compounds. Nevertheless, the structure of the group at C-4 strongly influences the activity. However, the theoretical results indicated that the intra-ring (C-1 and C-9) and π-exocycle (C-8 and C-14) carbons of caryophyllene-type structures promote the trypanocidal activity of these compounds.

Keywords: *Lychnophora pinaster*; DFT calculation; caryophyllene derivatives; trypanocidal activity; *Trypanosoma cruzi*.

Introduction

Chagas' disease (American trypanosomiasis) is a major cause of cardiomyopathy in South America. During the last years, the search for new chemical strategies to eliminate bloodstream forms of *Trypanosoma cruzi* has led to several compounds with trypanocidal activity [1], such as anfotericin B, tricomicin, β-carbolinic alkaloids, flavonoids, naphthoquinones, sesquiterpene lactones, triterpenes, and steroids [1-4]. Although some of them show *in vitro* and *in vivo* activities, they often can not be used, because they present low solubility in water or toxicity in active doses [5]. Gentian violet is able to kill *T. cruzi* trypomastigotes and sterilize blood at 4 °C, but there are some restrictions to its use, for instance potential mutagenicity and microaglutination [6]. Additionally, gentian violet changes blood color and this fact promotes its rejection by patients [7].

In the search for trypanocidal compounds, an extensive *in vitro* screening of the crude ethanol extract of Asteraceae species was carried out [8]. *Lychnophora* species extracts in

water, ethanol or "cachaça" (sugar cane spirit) are used in Brazilian folk medicine as analgesic, anti-inflammatory and anti-rheumatism remedies [9]. From one of them, *Lychnophora pinaster* Mart, *E*-lychnophoric acid, bicycle[7.2.0]undec-4-en-4-carboxylic acid-11,11-dimethyl-8-metilen-[1*R*-(1*R**,4*E*,9*S**)] **1** (Figure 1) was isolated [10-12]. This compound was submitted to anti-HIV, cytotoxicity by brine shrimp lethality model, and anti-tumor activity tests without major results [10]. However, **1** killed 100% of trypomastigote form of *T. cruzi* without promoting hemolysis.

The carboxylic group in **1** raises the question about the possible influence of the chemical function at C-4 on the trypanocidal activity of the caryophillene-type structures. In order to identify the major trypanocidal substances, two *E*-lychnophoric acid derivatives were prepared in this work: *E*-lychnophoric acid methyl ester **2**, methyl bicycle[7.2.0]undec-4-carboxylate-11,11-dimethyl-8-[1*R**(1*R**,4*E*,9*S**)], and the alcohol **3**, bicycle[7.2.0]4-undec-11,11-dimethyl-8-[1*R**(1*R**,4*E*,9*S**)] methanol (isocaryophyllen-15-ol) (Figure 1). Comparative analyses between chemical properties and trypanocidal activity *in vitro* were carried out to investigate the effect of the structure of the substituent group at C-4 on the potential activity of **1**, **2**, and **3**. The chemical properties of these compounds were obtained from theoretical calculations by DFT/BLYP/6-31G* method of most favored geometries in their conformational analyses.

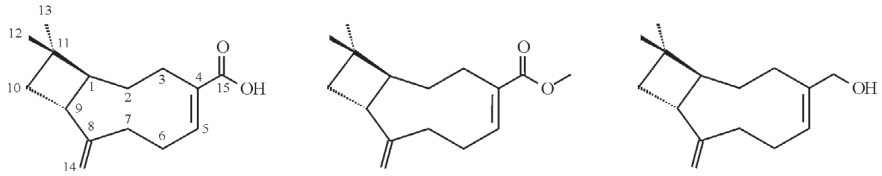

Figure 1. Lychnophoric acid **1**, its derivative ester **2** and alcohol **3**.

Experimental

*Preparation of **1** and its derivatives*

Thin Layer Chromatography for analytical (TLC) and preparative (PTLC) purposes was carried out on silica gel G. Anisaldehyde/H_2SO_4 was used as spraying reagent. GC-MS was performed using a Hewlett-Packard HP5890 Series 2 gas chromatograph coupled to a mass spectrometer HP5989A. The analyses were carried out in duplicate using a BP-5 column. Analysis conditions: column temperature gradient: 150 °C (1 min) and 290 °C (10 min); injection volume: 2 µL of hexane solution. The temperature of the injector was the same of the detector: 300 °C. IR spectra were measured in a KBr disc using a Shimadzu IR-408 apparatus. NMR spectra (400 MHz) were measured using a Bruker AM-400, and TMS as internal standard.

E-Lychnophoric acid (**1**), used as a raw material, was obtained as previously described [12]. IR (KBr, cm^{-1}) ν: 2400-2300, 2900, 2800, 1680, 1600, 1400, 1255 and 855; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 6.99 (t, *J*=7.9 Hz, H-5), 4.92 (d, *J*=0.9 Hz, H-14a), 4.80 (d, *J*=0.9 Hz, H-14b), 2.50 (dd, *J*=9.5 and 2.7 Hz, H-7a), 2.47 (dd, *J*=3.9 and 2.7 Hz, H-3a), 2.45 (ddd, *J*=5.4, 1.3 and 0.7 Hz, H-9), 2.42 (ddd, *J*=9.5, 2.0 and 1.3 Hz, H-7b), 2.36 (d, *J*=2.7 Hz, H-3b), 2.33 (ddd, *J*=5.4, 1.3 and 0.9 Hz, H-1), 2.27 (dd, *J*=7.9 and 2.0 Hz, H-6a), 1.80 (ddd, *J*=7.9 and 2.7 and 1.3 Hz, H-6b), 1.73 (dd, *J*=9.5 and 1.3 Hz, H-10a), 1.65 (ddd, *J*=15.5, 2.7 and 1.3 Hz, H-2a), 1.55 (dd, *J*=9.5 and 0.7 Hz, H-10b), 1.45 (ddd, *J*=15.5, 3.9 and 0.9 Hz, H-2b), 1.00 (s, H-13) and 0.98 (s, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.30 (C-15), 154.50 (C-8), 144.78 (C-5), 132.19 (C-4), 111.41 (C-14), 51.98 (C-1), 40.22 (C-10), 40.14 (C-9), 33.93 (C-6), 33.27

(C-11), 29.68 (C-13), 28.55 (C-7), 27.33 (C-2), 23.77 (C-3), and 22.88 (C-12); GC (RT): 4.42 min, MS (EI, 70 eV) *m/z*: 234 (M^+), 233, 219, 189, 174, 149, 147, 133, 119, 91 and 69.

E-Lychnophoric acid methyl ester **2** was obtained by the esterification of acid **1** with an acetic anhydride/pyridine mixture [13]. Briefly, acid **1** (7.7 mmol) was dissolved in acetone and added with K_2CO_3 and dimethyl sulphate to a mixture of acetic anhydride/pyridine. The reactional mixture was refluxed and the reaction was monitored by TLC. Usual work-up followed by silica gel column purification afforded ester **2** (yield 87%) as a pale oil. IR (KBr, cm^{-1}) ν: 2910, 2800, 1710, 1600, 1450, 1250, 1150, 1060 and 850; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.85 (t, *J*=7.9 Hz, H-5), 4.85 (d, *J*= 0.9 Hz, H-14a), 4.80 (d, *J*= 0.9 Hz, H-14a), 3.52 (s, CH_3-O), 2.50 (dd, *J*=9.5 and 2.6 Hz, H-7a), 2.47 (d, *J*=3.9 and 2.7 Hz, H-3a), 2.45 (dd, *J*=5.4 and 1.3 Hz, H-9), 2.42 (dd, *J*=9.5, 2.0 and 1.3 Hz, H-7b), 2.36 (d, *J*=2.7 Hz, H-3b), 2.33 (dd, *J*=5.4 and 1.3 Hz, H-1), 2.27 (dd, *J*=7.9 and 2.0 Hz, H-6a), 1.80 (ddd, *J*=7.9, 2.6 and 1.3 Hz, H-6b), 1.73 (dd, *J*=9.5 and 1.3 Hz, H-10a), 1.65 (ddd, *J*=15.5, 2.7 and 1.3 Hz, H-2a), 1.55 (d, *J*=9.5 Hz, H-10b), 1.45 (dd, *J*=15.5 and 3.9 Hz, H-2b), 1.00 (s, H-13) and 0.98 (s, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.35 (C-15), 154.76 (C-8), 141.98 (C-5), 132.71 (C-4), 111.29 (C-14), 52.05 (C-1), 51.57 (CH_3-O), 40.28 (C-10), 40.10 (C-9), 34.11 (C-6), 33.26 (C-11), 29.97 (C-13), 28.55 (C-7), 27.35 (C-2), 24.05 (C-3), and 22.92 (C-12); GC (RT): 7.49 min, MS (EI, 70 eV) *m/z*: 248 (M^+), 233, 216, 189, 174, 149, 147, 133, 119, 91 and 69.

Isocaryophyllen-15-ol **3** was obtained from the reduction of ester **2** by $LiAlH_4$/THF [14]. Briefly, **2** (0.692 mmol) was dissolved in THF and carefully added to a suspension of $LiAlH_4$ in THF. The reactional mixture was refluxed and the reaction was monitored by TLC. Usual work-up followed by silica gel column purification afforded alcohol **3** (yield 68%) as a pale and viscous oil. IR (KBr, cm^{-1}) ν: 3450, 2910, 2860, 1600, 1450, 1350, 1050 and 900; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.52 (t, *J*=8.9 Hz, H-5), 4.83 (d, *J*=2.2 Hz, H-14a), 4.74 (d, *J*=2.2 Hz, H-14a), 4.02 (s, CH_3-O), 2.48 (d, *J*=8.9 Hz, H-7a), 2.30-2.10 (m, H-3, H-9, H-7b, H-1, H-6a), 1.85-1.40 (m, H-6b, H-10, H-2), 0.99 (s, H-13) and 0.98 (s, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.47 (C-8), 139.59 (C-5), 126.72 (C-4), 110.56 (C-14), 67.18 (C-15), 52.01 (C-1), 40.61 (C-10), 40.07 (C-9), 35.07 (C-6), 33.27 (C-11), 29.99 (C-13), 27.19 (C-7), 26.61 (C-2), 25.76 (C-3), and 22.84 (C-12); GC (RT): 5.6 min. MS (EI, 70 eV) *m/z*: 220, 219, 205, 189, 174, 149, 147, 133, 119, 91 and 69.

In vitro trypanocidal test

Test samples were dissolved in DMSO (0.2 mL) plus Krebs-Ringer-glucose (1.8 mL) and mixed with infected blood (0.2 mL). Control tubes with DMSO and gentian violet (125 μg/mL) were run in parallel. After incubation at 4 °C for 24 h, the suspensions were microscopically examined. Only samples that killed 100% of the parasites were considered active [8].

Comparative analyses of the trypanocidal activity (*TA*) were made based on the variation of the necessary concentration of **1**, **2** and **3** to kill 100% of trypomastigotes *in vitro*. For convenience, *TA* values of ester **2** and alcohol **3** were determined relative to the concentration of the acid **1**. Thus, the *TA* value was always considered to be the unity (*TA* = 1.00) for this compound.

Theoretical Methodology

Theoretical studies were carried out using the software packages TITAN [15] and Gaussian03W [16]. Geometries optimized by the semi-empirical AM1 [17] were used as an initial model for the optimizations by the Density Functional Theory (DFT) [18] with the functional BLYP [19, 20] and the 6-31G* [21-25] (BLYP/6-31G*) set of bases. The geometries obtained by theoretical calculations were characterized as true minimal energy only when all the calculated frequencies (PES) were positive, considering the absence of intermolecular interactions in the gaseous state.

Calculations (DFT/BLYP/6-31G*) of energy and atomic contributions (orbital population) of the occupied and the virtual molecular orbitals (MO's) were made after geometry optimization on the same calculation level. Calculations (DFT/BLYP/6-31G*) of atomic charge were made by the Mulliken method with unities in electrons.

Calculated chemical properties were appropriately presented with average values. These averages were determined from chemical property values of the conformers of **1**, **2**, and **3** that presented higher populations. Thus, the values of chemical properties of conformers with relatively small populations were disregarded.

Similar to the trypanocidal activity analysis, average values of chemical properties of **1**, **2** and **3** were presented a function of acid **1**. Thus, for this compound, the average values were considered to be the unity. At the point corresponding to ester **2**, a multiplying factor *X* was applied to make the average values of chemical properties equal to the *TA* value this compound. The same multiplying factor *X* was also used to establish the average values of chemical properties for alcohol **3.** The better congruence between average chemical property and *TA* values obtained for **3** must probably indicate a better relationship with the alleged chemical activity.

Results and Discussion

In vitro trypanocidal test

At the test conditions, **1**, **2** and **3** were able to kill 100% of bloodstream form *T. cruzi* at concentrations of 13.86, 5.68 and 6.48 μg/mL, respectively. Although the compounds had shown significantly different activities (**3** and **2** are about two times more active than **1**), they are chemically related. This fact suggests some influence of the groups at C-4 on the trypanocidal activity. By comparative analysis of trypanocidal activity between these compounds as a function of concentration for the activity of acid **1**, the *TA* relative values for **1**, **2** and **3** are 1.00, 2.44 and 2.13, respectively. Thus, for all cases, $\Delta TA = 2.13$ was considered as a congruence factor between biological activity and chemical properties.

Conformational analysis

Initially, conformational distribution calculations (AM1) for **1**, **2**, and **3** were made. The obtained geometries were submitted to geometry optimization calculations (DFT/BLYP/6-31G*). For each compound, four conformers **a**, **b**, **c**, and **d** were generated from these calculations as minimal energies. Figure 2 shows the geometries of the conformers of **1**, which are different from each other only in the skeleton conformation of the cyclononene ring.

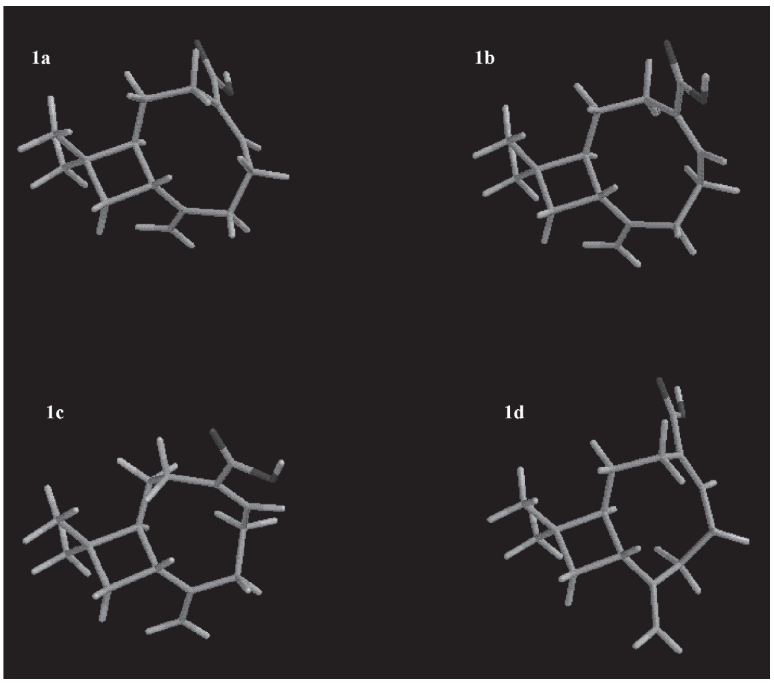

Figure 2. Geometries of conformers from **1a** to **1d** generated by the conformational search (AM1) and optimized by calculations (DFT/BLYP/6-31G*). Conformers corresponding to **2** and **3** present the same geometries of the cyclononen ring, showed in that figure for **1**

Table 1 presents the results of thermodynamic calculations (DFT/BLYP/6-31G*) of the conformers of **1**, **2** and **3**. For each compound, conformer **a** presents relatively lower enthalpy, with a value close to those of conformers **b** and **c**. The largest enthalpy of conformer **d** could indicate its smaller contribution to the conformational populations of **1**, **2**, and **3**. Nevertheless, in the case of structurally simple cycloalkanes, conformational analyses based only on enthalpy calculations, can differ from those experimentally observed [26-30]. Therefore, the conformational population of these caryophillene-type structures can be better investigated by enthalpy and free energy calculations. According to Table 1, the enthalpy variation between the conformers of each compound is not very significant. The results in free energy values follow the same order of stability of the conformers proposed by enthalpy.

For cycloalkanes, conformational stability depends on the total tension of the ring, which is made up of angular, torsional and bond tensions, as well as van der Waals compressions. In medium rings (C_{8-11}), van der Waals compressions are due mainly to transannular tensions involving hydrogen atoms of adjacent methylene groups. When determined experimentally, the total tension of the cyclononane in relation to the *n*-nonane is of the order of 12.6 kcal/mol [31]. In many cases, the transannular tensions can significantly influence the conformational stability of cyclic structures similar to those of caryophyllene-type structures. Theoretical studies carried out by Molecular Mechanics show that the angular deformations caused by transannular tensions in cyclononanes favor geometries with bond angles of 124 degrees [31, 32].

The average angles (θ_{av}) of the cyclononene C-C-C bonds obtained by DFT/BLYP/6-31G* optimized geometries of the conformers of **1**, **2** and **3** are presented in Table 1. For each compound, conformer **a**, which presents the lowest energy, also has the smallest value of θ_{av} (≈ 116.7 degrees). In contrast, conformer **d**, which presents the highest energy, has a larger value of θ_{av} (≈ 119.1 degrees). These results demonstrate a relation between the enthalpy and the θ_{av} angles in cyclononane rings. Calculations (DFT/BLYP/6-31G*) of geometry optimization of non-substituted cyclononane were performed and gave a value of θ_{av} (116.20 degrees) very close to those of conformers **a** (Table 1). Therefore, smaller van der Waals compressions are expected for **1a**, **2a** e **3a**.

As in Table 1 the values of ΔG of conformers **a**, **b**, and **c** of each compound are very close and significantly smaller in relation to those of conformer **d** (≈ 7.6 kcal/mol). It is expected that its contribution to the conformational distribution of **1**, **2**, and **3** is not significant.

Table 1. Calculated values (DFT/BLYP/6-31G*): enthalpy (ΔH_f), entropy (ΔS) and free energy (ΔG), as well as average angles (θ_{av}) of cyclononen C–C–C bonds of the conformers **a**, **b**, **c** and **d** of **1**, **2** and **3**

Conformer	ΔH_f (kcal/mol)	ΔS (cal/mol K)	ΔG (kcal/mol)	θ_{av} (degrees)
1a	-81.52	127.46	-119.52	116.66
1b	-80.19	127.72	-118.27	117.47
1c	-79.50	127.53	-117.52	117.46
1d	-74.22	126.26	-111.86	119.06
2a	-74.73	134.69	-114.89	116.67
2b	-73.40	135.42	-113.78	117.47
2c	-72.68	134.97	-112.92	117.46
2d	-67.44	133.65	-107.29	119.07
3a	-43.89	124.31	-80.95	116.69
3b	-40.79	125.42	-78.18	117.48
3c	-39.95	125.44	-77.35	117.43
3d	-36.49	123.32	-73.26	119.14

[#] 298.15 K and 1 atm

Chemical property calculations and comparison with biological activity

Initially, theoretical calculations (DFT/BLYP/6-31G*) of energy of the frontier molecular orbitals (MO_f's) of **1**, **2**, and **3** were performed for conformers **a**, **b** and **c**. Table 2 shows calculated average energies (DFT/BLYP/6-31G*) of occupied and virtual frontier molecular orbitals of the conformers of **1**, **2** and **3** and comparative analyses about these energies as a function of the corresponding values obtained for **1**. By comparison between the absolute values of MO energies in Table 2, the energies of the occupied virtual MO's of **1** and **2** are very close when compared to the corresponding energies of **3**. A similar behavior was observed for the energies of the virtual MO's of these compounds. In this way, calculated energy variations do not correspond to the closer trypanocidal activity observed *in vitro* for **2** and **3**. When compared to the value obtained for **1**, the energy relations closer to ΔTA = 2.13 (correspondent *TA* value to **3**) in Table 2 were only verified in the LUMO+3, LUMO+4 and LUMO+5, being 2.42, 2.60 and 2.82, respectively. For other MO's, more different relations to 2.13 were verified. Therefore, the character either nucleophilic or electrophilic given by the energies of occupied and virtual MO's, respectively, do not allow establishing relations with trypanocidal activity of these compounds.

The experimental infrared spectra of **1**, **2**, and **3** show weak absorptions and overlapping in the region between 1750 and 1550 cm^{-1}. For this reason, the analyses of these spectra do not allow the correlation of the effect of the group at C-4 on the experimentally observed vibrational frequencies of the π bonds. Vibrational calculations (DFT/BLYP/6-31G*) were carried out for the conformers **a**, **b**, and **c** of these compounds. Two vibrations can be attributed to the stretching of the C-1–C-9 bond, which presents high annular tension characteristic of the cyclobutane ring. One vibrations were verified for **1**, **2**, and **3** (ν_1, ν_2 and ν_3, respectively) at ν_1=1536.0, ν_2=1.536.0 and ν_3=1.536.2 cm^{-1} and another at ν_1=1469.6, ν_2=1469.7 and ν_3=1.469.8 cm^{-1}. Calculated stretching frequencies of the exocyclic π bond (C-8–C-14) in **1**, **2** and **3** are ν_1=1870.3, ν_2=1870.4 and ν_3=1870.3 cm^{-1}, respectively. In these cases, it is observed that the calculated frequencies do not suffer significant variations as a function of the structure of the group at C_4.

For the C-4–C-5 π endocyclic bond, the calculated frequencies are ν_1=1896.3 cm^{-1}, ν_2=1898.1 cm^{-1}, and ν_3=1916.3 cm^{-1}. Even though the frequencies ν_1 and ν_2 are very close ($\Delta\upsilon \approx 1.8$ cm^{-1}), both differ significantly from ν_3 ($\Delta\nu \rangle 18$ cm^{-1}). This fact demonstrates an effect of the substituent in C-4 on the vibrational frequencies of the endocyclic π bond. However, these frequencies do not present a variation that corresponds to the experimentally observed trypanocidal activity.

In comparison to the frequencies of the π bonds, the exocyclic π bond presents relatively smaller values. This observation indicates a smaller force constant for this bond, and thus, a greater lability in relation to the endocyclic π bond. Therefore, these results suggest a larger chemical reactivity of the π exocyclic bond, which can be involved in the potential activities of these compounds.

Table 3 shows the calculated average of Mulliken atomic charge (DFT/BLYP/6-31G*) on the conformers of **1**, **2** and **3**, and comparative analyses as a function of the corresponding value obtained for **1**. In the C-1–C-9 bond, the calculated atomic charges (*q* in au) on C-1 of **1**, **2** and **3** ($q_{1(1)}$ = -0.071; $q_{1(2)}$ = -0.069; $q_{1(3)}$ = -0.046) and on C-9 ($q_{9(1)}$ = -0.156; $q_{9(2)}$ = -0.152; $q_{9(3)}$ = -0.172) showed very close absolute values between **1** and **2**. In Table 2, charge relations on C-1 ($\Delta q_{C\text{-}1}$ = 1.63) and C-9 ($\Delta q_{C\text{-}9}$ = 2.76) presented significant variation when compared to *TA* relations of these compounds (ΔTA = 2.13).

In π bonds, calculated charges on C-4 ($q_{C\text{-}4(1)}$ = 0.174; $q_{C\text{-}4(2)}$ = 0.159 and $q_{C\text{-}4(3)}$ = 0.268) and C-5 ($q_{C\text{-}5(1)}$ = -0.121; $q_{C\text{-}5(2)}$ = -0.127 and $q_{C\text{-}5(3)}$ = -0.152) indicate significant variations due to the structure of the group at C-4. Table 3 shows comparative values of calculated charges on C-4 ($\Delta q_{C\text{-}4}$ = 4.11) and C-5 ($\Delta q_{C\text{-}5}$ = 2.92) with more significant variation in relation to the biological activity (ΔTA = 2.13) that corresponding to C-1 and C-9. On the other hand, calculated charges on C-8 ($q_{C\text{-}8(1)}$ = 0.282; $q_{C\text{-}8(2)}$ = 0.299 and $q_{C\text{-}8(3)}$ = 0.290) and C-14 ($q_{C\text{-}14(1)}$ = -0.299; $q_{C\text{-}14(2)}$ = -0.245 and $q_{C\text{-}14(3)}$ = -0.262) correspond to $\Delta q_{C\text{-}8}$ = 2.37 and $\Delta q_{C\text{-}14}$ = 2.60. These relations are very close to ΔTA = 2.13 and suggest the involvement of the π

exocyclic system in the trypanocidal activities of these compounds. As demonstrated by the larger atomic charge on C-14 in relation to C-8 ($q_{14} \gg q_8$), if there are effects of electrostatic interactions of the π exocyclic system on the biological activity, the electrophilic character of C-8 and/or the nucleophilic character of C-14 must be considered.

Table 3 list the DFT/BLYP/6-31G* calculated average orbital population for occupied (Δc_o) and virtual (Δc_v) MO's of **1**, **2**, and **3** as function of **1**, considering only the results closer to Δ*TA*. The larger congruencies in relation to the Δ*TA* are observed in Δc_o of C-8 to HOMO and HOMO-3 (Δc_o = 2.07 and 0.76, respectively) and C-1 to HOMO-4 and HOMO (Δc_o = 2.20 and 1.37, respectively). For virtual MO's, the larger congruencies in relation to the Δ*TA* are only observed in Δc_v of C-1 to LUMO+3 (Δc_v = 2.74) and C-9 to LUMO+5 and LUMO+4 (Δc_o = 2.75 and 2.76, respectively). Thus, the results of these calculations suggest a more significant involvement of both the exocyclic π system and the cyclobutane ring in the trypanocidal activity of these caryophyllene compounds.

Table 2. Calculated average energies (DFT/BLYP/6-31G*) of occupied and virtual frontier MO's between conformers of **1**, **2** and **3**, and comparative analyses as function of the correspondent values of **1**[a]

MO	Energy (eV)			Comparative analyses		
	1	**2**	**3**	**1**[b]	**2**[b]	**3**[d]
LUMO+5	1.630	1.798	2.065	1.00	2.44	2.80
LUMO+4	1.481	1.569	1.674	1.00	2.44	2.60
LUMO+3	1.363	1.424	1.401	1.00	2.44	2.42
LUMO+2	1.141	1.046	1.260	1.00	2.44	2.92
LUMO+1	-0.153	-0.075	0.289	1.00	2.44	-9.41
LUMO	-1.640	-1.503	0.102	1.00	2.44	-0.16
HOMO	-5.308	-5.282	-8.715	1.00	2.44	4.04
HOMO-1	-5.549	-5.525	-9.004	1.00	2.44	3.99
HOMO-2	-5.870	-5.787	-9.480	1.00	2.44	3.97
HOMO-3	-6.322	-6.271	-10.331	1.00	2.44	4.02
HOMO-4	-6.516	-6.356	-10.483	1.00	2.44	4.01
HOMO-5	-6.750	-6.426	-10.917	1.00	2.44	4.16

(a) Comparative analyses about *TA* values, Δ*TA* = 2.13; (b) energy of **1**/energy of **1**; (c) [(energy of **2**/energy of **1**) *x* 2.44]/(energy of **2**/energy of **1**); (d) [(energy of **3**/energy of **1**) *x* 2.44]/(energy of **2**/energy of **1**).

Table 3. Calculated average of atomic charge (Δq) and orbital populations (DFT/BLYP/6-31G*) of occupied (Δc_o) and virtual (Δc_v) frontier molecular orbitals of the conformers of **1**, **2** and **3** by comparative analyses as function of the corresponding values obtained for **1**

	Compound				
	1	**2**	**3**		
Entry			Δq	Δc_o	Dc_v
1	1.00	2.44	2.37 ($q_{C\text{-}8}$)	2.07 ($C_{C\text{-}8}$ to HOMO)	2.74 ($C_{C\text{-}1}$ to LUMO+3)
2	1.00	2.44	2.60 ($q_{C\text{-}14}$)	2.20 ($C_{C\text{-}1}$ to HOMO-4)	2.75 ($C_{C\text{-}9}$ to LUMO+5)
3	1.00	2.44	1.63 ($q_{C\text{-}1}$)	1.37 ($C_{C\text{-}1}$ to HOMO)	2.76 ($C_{C\text{-}9}$ to LUMO+4)
4	1.00	2.44	2.76 ($q_{C\text{-}9}$)	0.76 ($C_{C\text{-}8}$ to HOMO-3)	3.27 ($C_{C\text{-}9}$ to LUMO+2)
5	1.00	2.44	2.92 ($q_{C\text{-}5}$)	0.72 ($C_{C\text{-}9}$ to HOMO-3)	0.91 ($C_{C\text{-}14}$ to LUMO+5)
6	1.00	2.44	4.11 ($q_{C\text{-}4}$)	-0.55 ($C_{C\text{-}14}$ to HOMO)	-0.25 ($C_{C\text{-}14}$ to LUMO+4)

Conclusions

According to the trypanocidal tests carried out, the alteration of caryophillene structure due to the substituent group in C-4 can affect the potential activity of **1**, **2**, and **3**. The geometry optimization calculations (DFT/BLYP/6-31G*) do not show any effect of these substituents in the conformational analysis of the caryophillene skeleton. Thus, the trypanocidal action of **1**, **2** and **3** cannot be related to the spatial changes on the caryophillene skeleton given by the different substituent groups in C-4.

However, theoretical calculations show an effect of the substituent on the chemical properties related to the C-1–C-9 bond and the exocyclic π (C-8–C-14) and the endocyclic π (C-4–C-5) systems. Comparing the results of the trypanocidal test with the chemical properties calculated for **1**, **2**, and **3** lead us to conclude that the activity may be determined by the chemical properties of the exocyclic π bond and the C-1–C-9 bond of both the cyclobutane ring. Despite the spatial proximity of the substituent group to the endocyclic π system, the theoretical calculations performed did not allow an inference about the relation between the chemical properties of this π system and the trypanocidal activities of **1**, **2**, and **3**.

Acknowledgement

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo a Pesquisa do Estado do Amazonas (FAPEAM), and Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

Supplementary Material

Tables with all optimized geometrical parameters and other results for all structures considered in the present work are available from the authors upon request.

Recebido em: 08/04/2005
Aceito em: 28/07/2005

A. F. C. Alcântara, D. Silveira, E. Chiari, A. B. Oliveira, J. E. Guimarães, D. S. Raslan. Análise comparativa, por cálculos teóricos, da atividade tripanossomicida e das propriedades químicas do ácido *E*-licnofórico e de seus derivados.

Resumo: O ácido *E*-licnofórico **1**, seus derivados éster **2** e o álcool **3** eliminaram 100% das formas tripomastigota de *Trypanosoma cruzi* nas concentrações de 13,86, 5,68 e 6,48 μg/mL, respectivamente. Cálculos (AM1) de distribuição conformacional de **1**, **2** e **3** resultaram somente em quatro confôrmeros **a**, **b**, **c** e **d** como mínimos de energia, que diferem entre si apenas na geometria do anel ciclononeno. Cálculos (DFT/BLYP/6-31G*) de otimização de geometria e de propriedades químicas foram realizados para os confôrmeros de **1**, **2** e **3**. Esses resultados teóricos foram comparados numericamente com os resultados de atividade tripanossomicida. Valores calculados de densidade eletrônica e cargas atômicas, população orbital e freqüências vibracionais mostraram que o sistema π-endocíclico (C-4 e C-5) não é um sítio determinante dessa atividade. Entretanto, a estrutura do grupo oxigenado influencia fortemente o potencial de outros sítios do esqueleto cariofilênico. Assim, os cálculos sugerem que o sistema π-exocíclico (C-8 e C-14) e os carbonos C-1 e C-9 promovem a atividade tripanossomicida dessas substâncias.

Palavras-chave: *Lychnophora pinaster*; DFT; derivados cariofilênicos; atividade tripanossomicida, *Trypanosoma cruzi*

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