

Lychnophoric acid from *Lychnophora pinaster*: a complete and unequivocal assignment by NMR spectroscopy.

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Abstract: The investigation of the hexane extract from aerial parts of *Lychnophora pinaster* provided, besides others substances, the *E*-isomer of lychnophoric acid, a sesquiterpene derivative previously isolated from *L. affinis*.

Keywords: *Lychnophora pinaster*; Asteraceae; lychnophoric acid.

Introduction

Plant species of the genus *Lychnophora* (*Asteraceae*) are known as “candeia”, “arnica” and “arnica da serra” and are used in folk medicine as anti-flogistic, anti-rheumatic, and analgesic [1]. Typical constituents of *Lychnophora* species are sesquiterpene lactones [2] of which 15-deoxygoyazensolide was shown to be active against *Trypanosoma cruzi*, the etiological agent of Chagas’ disease (American trypanosomiasis) [3]. Prompted by this observation we have carried out a screening of *Asteraceae* plant species in the search of new trypanocidal agents [4] and we have investigated three active *Lychnophora* species, one of them being *L. pinaster* Mart. Bioguided fractionation of the hexane and dichloromethane extracts of the aerial parts of this plant [5] led to the isolation of lychnophoric acid (*I*), previously isolated from *L. affinis*, that was assayed *in vitro* against bloodstream forms of *T. cruzi* and presented 50% growth inhibition in the dose of 12,0mg/mL [6].

Experimental

General

Melting point was determined on a Mettler FP5 apparatus; $[\alpha]_D^{25}$ was measured at 25 °C on a Bellincham & Stanley Ltd P-20 polarimeter. IR spectrum was obtained on a Shimadzu/IR-408 spectrometer. EIMS was obtained on a Kratos MS 80 RFA spectrometer. ¹H and ¹³C NMR spectra and contour plots were acquired on a Bruker AVANCE DRX400 instrument operating at 400 MHz for ¹H and 100 MHz for ¹³C. HPLC analysis was performed with a Shimadzu CR-8, UV detector. CG analysis was performed with a HP5890 gas chromatograph, FID detector, and a VDC3390A integrator.

Plant material

The aerial parts of *Lychnophora pinaster* Mart. were collected at Serra da Moeda, State of Minas Gerais, Brazil, in March 1992. A voucher specimen has been deposited in the Herbarium of

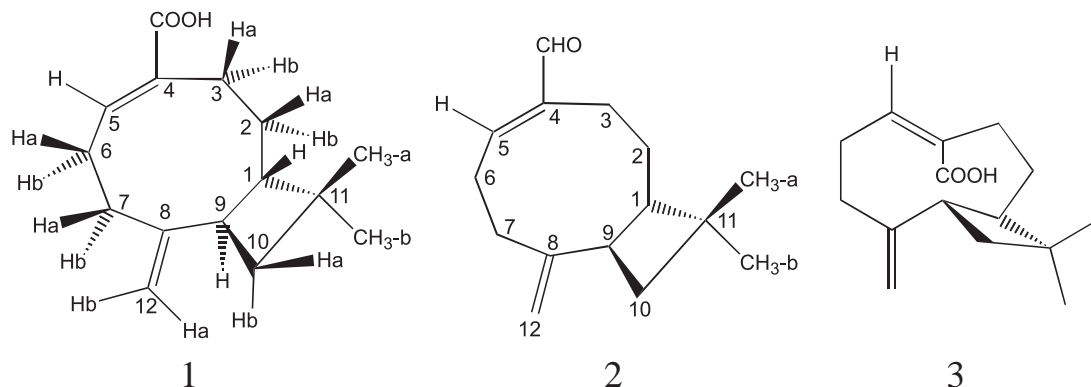
the Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Minas Gerais (BHCB-UFMG 19520).

Extraction procedures

The dried aerial parts (2.0 Kg) were powdered and successively extracted with n-hexane and dichloromethane. The solvents were removed under vacuum, below 40 °C, to give 114.0 g of n-hexane and 12.0 g of dichloromethane extracts.

The crude extracts were chromatographed first by CC (Silica gel 60, hexane-CH₂Cl₂-AcOEt-MeOH gradient). The n-hexane extract (114.0 g) furnished a homologue series of saturated hydrocarbons (C₂₂-C₃₂) [7], lupeol, a- and b-amyryn, friedelin and fat acid esters detected by GC, in comparison with authentic samples. The CH₂Cl₂ fr. was chromatographed over florisil column. Fraction 1 (petrol), after washing with Et₂O-MeOH (1:1), filtration and solvent evaporation, afforded a yellow gum, which was partitioned between hexane and MeOH-H₂O (9:1). The MeOH-H₂O fr., after 4 days at 4 °C, afforded *I*. CC of the CH₂Cl₂ extract (12.0 g) afforded a homologue series of saturated hydrocarbons (C₂₅-C₃₂) [7] detected by GC, as well quercetin and 15-deoxygoyazensolide, detected by HPLC, using authentic samples as standard.

E-Lychnophoric acid (*1*): Bicyclo [7.2.0] undec-4-en-4-carboxylic acid-11,11-dimethyl-8-methylen-[1*R*-(1*R**,4*E*,9*S**)]. Amorphous solid, mp 118-9 °C (Et₂O), [α]_D²⁰ = -24° (CHCl₃; c = 0,054). IR ν_{\max} cm⁻¹ 3050-2400, 2900, 1680 (C=CCO₂H); 1640 (C=CH₂), 890. EIMS *m/z* (rel. int.): 254 [M⁺] (15) (C₁₅H₂₂O₂), 219 [M-Me] (25), 69 (C₅H₉⁺) 100. ¹H NMR and ¹³C NMR (see Table 1).



Quercetin: R_t = 17.16 min. HPLC conditions: LiChroCART 125-4 RP-18 column; MeCN/H₂O gradient, 15 to 45%, 30 min.

15-deoxygoyazensolide: R_t = 9.19 min. HPLC conditions: LiChroCART 125-4 RP-18 column; Hexane-CH₂Cl₂ (3:7) isocratic, 0.5 mL/min.

Results and discussion

The hexane extract from the dried aerial parts of *L. pinaster* was column chromatographed over silica gel affording mixtures of homologue hydrocarbons [7], triterpenes (lupeol, a- and b-amyryn, friedelin), fat acids (identified by GLC of their methyl esters), and a caryophyllene derivative, lychnophoric acid (*1*). The dichloromethane extract afforded a mixture of homologue hydrocarbons. Quercetin and 15-deoxygoyazensolide were detected by HPLC in comparison with authentic samples.

The IR spectrum of compound *1* showed absorption bands due to conjugated carboxylic function group (3600-2400, 1680 cm⁻¹), carbon-carbon double bonds (1640, 1470, 890 cm⁻¹), and *gem*-dimethyl groups (1370 cm⁻¹). Its ¹H NMR spectrum (Table 1) exhibited characteristic signals indicating the presence of a terminal olefinic methylene group (δ 4.87 and δ 4.81) and another olefinic hydrogen in an a,b-unsaturated carboxylic group (δ 7.00). Two 3H singlets at δ 0.96 and δ 1.00 confirmed a *gem*-dimethyl group. EIMS indicated a [M]⁺ of *m/z* 254, which in conjunction with ¹H and ¹³C NMR data allowed the assignment of the molecular formula C₁₅H₂₂O₂ to (*1*). These data are very similar to those reported for lychnophoric acid (*3*) [8,9].

Table 1: NMR* data (δ) from lychnophic acid (1), Isocaryophyllen-13-al (2) and lychnophoric acid (3).

| | 1 | 2 [10] | 3 [8] | 1 | 2 [10] | 3 [9] |
|-----------|-----------------------------------|--------------------|-----------------------|-------------------------|-------------------------|---------------------|
| 1 | 1.81 (ddd) | 1.70 | 1.65 (m) | 51.90 | 52.38 | 52.10 |
| 2 | a:1.48 (dddd); b:1.67 (ddt) | 1.44; 1.65 | 1.45 (m) | 27.30 | 27.10 | 27.40 |
| 3 | 2.33 (m); 2.43 (m) | 2.39; 2.28 | 2.25 (m) | 23.70 | 21.89 | 23.70 |
| 4 | - | | - | 132.00 | 144.20 | 132.20 |
| 5 | 7.00 (t [†]) | 6.54 | 6.22 (m) | 144.70 | 154.46 | 144.70 |
| 6 | 2.30 (m); 2.41 (m) | 2.65; 2.36 | 2.25 (m) | 33.90 | 28.81 | 34.00 |
| 7 | 2.41 (m); 2.50 (m) | 2.50; 2.36 | 2.33 (m) | 28.50 | 34.19 | 28.50 |
| 8 | - | - | - | 154.50 | 153.94 | 154.40 |
| 9 | 2.50(q [‡]) | 2.43 | 2.65 (m) | 40.10 | 40.97 | 40.20 |
| 10 | a:1.73(ddd); b:1.57(dd) | a: 1.57; b:1.71 | 1.65 (m) | 40.20 | 40.04 | 40.30 |
| 11 | - | - | - | 33.20 | 33.48 | 33.30 |
| 12 | a: 4.87(dd); b: 4.81(m) | a:4.86; b:4.82 | 5.03 (d); 4.88 (d) | 111.40 | 111.64 | 111.50 |
| Me | a:1.00(s); b:0.96(s) | a:0.94; b:0.98 | 1.02 (s); 1.00 (s) | a:29.90 ; b:22.80 | a:30.04 ; b:22.73 | a:30.00; b:22.90 |
| CO | - | - | - | 173.00 | 195.48 | 173.80 |

1: 400MHz (¹H); 100MHz(¹³C); 2: 500MHz (¹H); 125MHz(¹³C); 3: 200MHz (¹H); 50MHz (¹³C);* TMS as internal standard; [†] apparent triplet; [‡] apparent quartet;

Coupling Constants (Hz): In parentheses are the analogous values for 2 and 3, respectively. $J_{1,2a} = 12.0$; $J_{1,2b} = 3.8$; $J_{1,9} = 9.2$; $J_{1,10a} = 0.7$; $J_{2a,2b} = 13.9$; $J_{2a,3a} = 7.6$; $J_{2a,3b} = 12.0$; $J_{2b,3a} = 9.1$; $J_{2b,3b} = 3.8$; $J_{5,6a} = 7.8$; $J_{5,6b} = 9.3$; $J_{9,10a} = 9.4$; $J_{9,10b} = 9.4$; $J_{10a,10b} = 10.9$; $J_{12a,7a}$ and $J_{12a,7b} = 1.6$ or 0.8 .

However, divergences between **1** and **3** were observed for the ^1H NMR data: the signal of H-5 is shifted to a higher value of δ 7.00 in the former, in comparison to that one originally described for lychnophoric acid (δ 6.22) [8]. This fact can be explained by the change in the configuration of the double bond from *Z*-configuration in **3** to *E*-configuration in **1**, where the closer carbonyl group can contribute with its stereoelectronic deshielding effect. Besides the difference in chemical shifts, a difference in the multiplicity of the H-5 signal in the two compounds is also observed. In the *Z*-isomer (**3**), this signal is described as a multiplet due to coupling with the two adjacent H-6 and to a long-range coupling with two allylic H-3 [8]. The *E*-isomer (**1**) ^1H NMR spectrum shows an apparent triplet (δ 7.00, $J=7.8$ Hz and $J=9.3$ Hz) for H-5 due to imperfect superposition of the two inner signals of the theoretical double doublet, and the long range

coupling with the two H-3 is not observed.

Despite the use of the Gaussian multiplication with Trafficante function altogether in the normal fid, we could not achieve enough improvement of resolution to picture the H-5 theoretical double doublet. Likewise for the compound **2**, the signal of H-9 appears as a quartet. All chemical shifts were supported by one and two-dimensional NMR techniques like NOEDIFF, COSY and NOESY. In particular the HMQC experiment was very important to the assignments of the chemical shifts inside the complex envelopes. For example, a strong nOe were observed for the protons H-12a (δ 4.87) with H-10b (δ 1.57), H-10a (δ 1.73), H-9 (δ 2.50) and Me-b group (δ 1.00) and between the protons H-9 (δ 2.50) and Me-a (δ 0.96) as well for the H-12b (δ 4.81) with H-7a,b spin system. The nOe were also observed for H-5 (δ 7.00) and H-6a,b system. The nOe results are summarized in the figure 1.

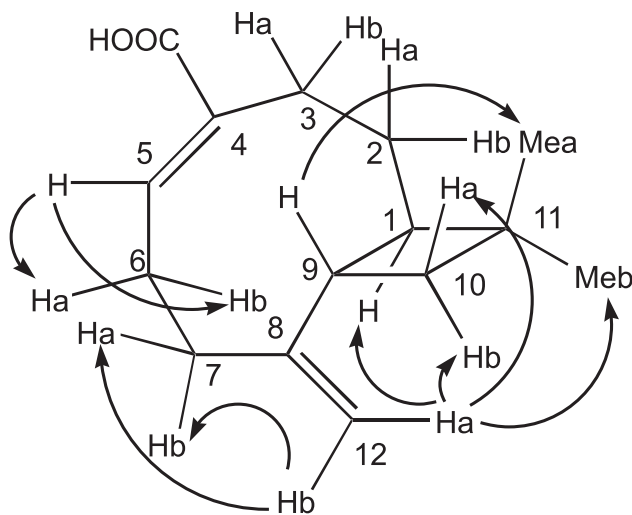


Figure 1. nOe assignments for lychnophoric acid (**1**) by NOESY experiment (ns 16, ds 4, d8 0.5 sec, TD 2K)

Conclusions

The ^{13}C NMR data for compound **1** are very close to those reported for compound **3** [9] (TABLE 1). The authors [9] did not report the ^1H NMR data.

These data led us to consider (**1**), is in fact the *E*-isomer of lychnophoric acid, originally described as the *Z*-isomer (**3**) in reference 8. Based on the reported ^{13}C NMR data (Table 1) the

compound reported also represents the *E*-isomer (**1**) instead of the *Z*-isomer (**3**), as previously proposed [9].

The spectral data and nOe results of **1** are in good accord with data reported for aldehyde **2** [10].

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D. Silveira, J. D. de Souza Filho, A. B. de Oliveira, D. S. Raslan. Atribuição completa e inequívoca dos sinais de deslocamento químico dos átomos de carbono e hidrogênio do ácido licnofórico extraído de *Lychnophora pinaster*.

Resumo: O estudo químico das partes aéreas do extrato hexânico de *Lychnophora pinaster* forneceu, além de outras substâncias, o isômero *E* do ácido licnofórico, um sesquiterpeno anteriormente isolado de *L. affinis*.

Palavras-chave: *Lychnophora pinaster*; Asteraceae; ácido licnofórico.

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