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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; lychnophoic 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(***1***), 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; $[a]_D$ 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 ^{13}C NMR spectra and contour plots were acquired on a Bruker *AVANCE* DRX400 instrument operating at 400 MHz for ^1H and 100 MHz for ^{13}C. 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-CH_2Cl_2-AcOEt-MeOH gradient). The n-hexane extract (114.0 g) furnished a homologue series of saturated hydrocarbons (C_{22} -C_{32}) [7], lupeol, a- and b-amyrin, friedelin and fat acid esters detected by GC, in comparison with authentic samples. The CH_2Cl_2 fr. was chromatographed over florisil column. Fraction 1 (petrol), after washing with Et_2O-MeOH (1:1), filtration and solvent evaporation, afforded a yellow gum, which was partitioned between hexane and MeOH-H_2O (9:1). The MeOH-H_2O fr., after 4 days at 4 °C, afforded ***1***. CC of the CH_2Cl_2 extract (12.0 g) afforded a homologue series of saturated hydrocarbons (C_{25} -C_{32}) [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-[1R-(1R,4E ,9S*)]*. Amorphous solid, mp 118-9 °C (Et_2O), $[a]_D^{20}$= -24° ($CHCl_3$; *c*= 0,054). IR n_{max} cm^{-1} 3050-2400, 2900, 1680 (C=CCO_2H); 1640 (C=CH_2), 890. EIMS *m/z* (rel. int.): 254 [M^+] (15) ($C_{15}H_{22}O_2$), 219 [M-Me] (25), 69 ($C_5H_9^+$) 100. ^1H NMR and ^{13}C NMR (see Table 1).

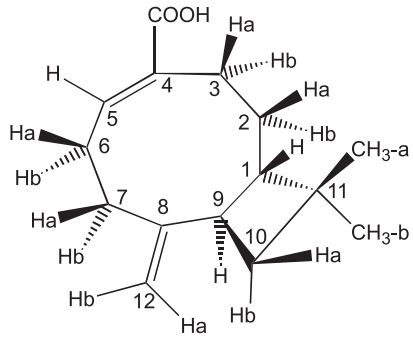
Quercetin: R_t = 17.16 min. HPLC conditions: LiChroCART 125-4 RP-18 column; MeCN/H_2O gradient, 15 to 45%, 30 min.

15-deoxygoyazensolide: R_t = 9.19 min. HPLC conditions: LiChroCART 125-4 RP-18 column; Hexane-CH_2Cl_2 (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-amyrin, 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 (d 4.87 and δ 4.81) and another olefinic hydrogen in an a,b-unsaturated carboxylic group (δ 7.00). Two 3H singlets at d 0.96 and d 1.00 confirmed a *gem*-dimethyl group. EIMS indicated a $[M]^+$ of m/z 254, which in conjunction with ^1H and ^{13}C NMR data allowed the assignment of the molecular formula $C_{15}H_{22}O_2$ to (***1***). These data are very similar to those reported for lychnophoric acid (***3***) [8,9].



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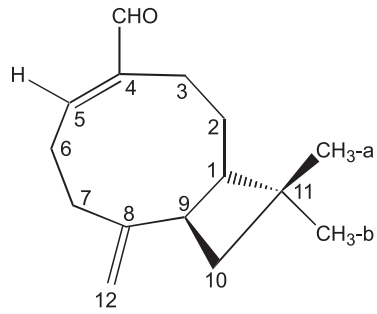

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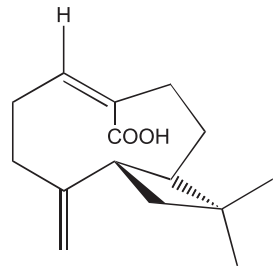

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Table 1: NMR* data (δ) from lychnophoic acid (*1*), Isocaryophyllen-13-al (*2*) and lychnophoric acid (*3*).

	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$^{\hat{I}}$)	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 (^{1}H); 100MHz(^{13}C); 2: 500MHz (^{1}H); 125MHz(^{13}C); 3: 200MHz (^{1}H); 50MHz (^{13}C);* TMS as internal standard; $^{\hat{}}$ 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 ^{1}H NMR data: the signal of H-5 is shifted to a higher value of d 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***) ^{1}H 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 Traficante 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.

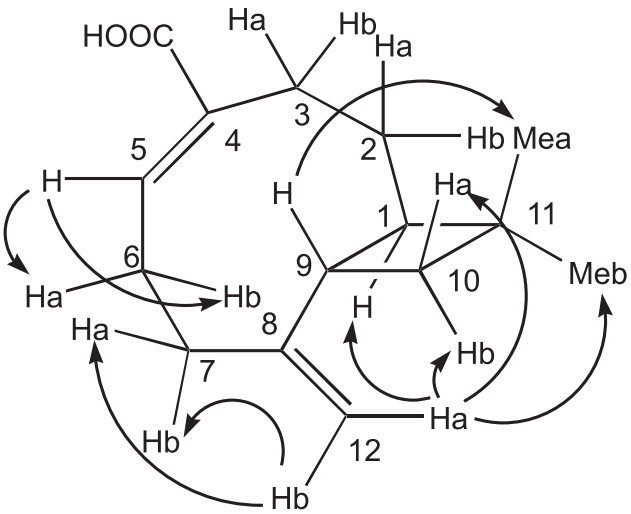

Figure 1. nOe assignments for lychnophoic acid (*1*) by NOESY experiment (ns 16, ds 4, d8 0.5 sec, TD 2K)

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 ^{1}H 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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