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

Silveira, D.; de Souza Filho, J. D.; de Oliveira, A. B.; Raslan, D. S.
Lychnophoric acid from Lychnophora pinaster: a complete and unequivocal assignment by NMR spectroscopy
Eclética Química, vol. 30, núm. 1, janeiro-março, 2005, pp. 37-41
Universidade Estadual Paulista Júlio de Mesquita Filho
Araraquara, Brasil

ECLÉTICA Química
www.scielo.br/eq
Volume 30, número 1, 2005

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

D. Silveira [1], J. D. de Souza Filho [2], A. B. de Oliveira [3], D. S. Raslan [2]*
[1]Faculdade de Ciências da Saúde, UnB Asa Norte, Brasília, DF, Brazil
[2]Departamento de Química, ICEx, UFMG. Av. Antônio Carlos 6627, CEP 31270-010. Belo Horizonte, MG, Brazil.
[3]Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, UFMG . Av. Olegário Maciel, 2360, CEP 30180-112. Belo Horizonte, MG, Brazil.
**To whom correspondence should be addressed; e-mail: damaris@unb.br*

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).

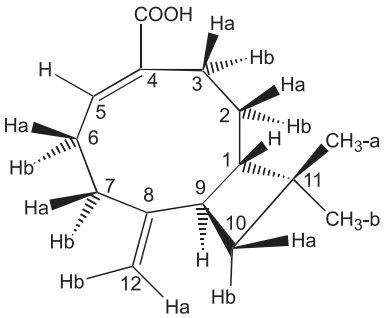
Quercetin: R_t = 17.16 min. HPLC conditions: LiChroCART 125-4 RP-18 column; MeCN/H_2O gradient, 15 to 45%, 30 min.

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.

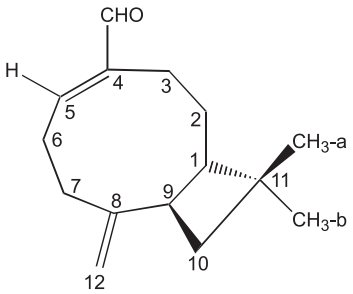
Results and discussion

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.

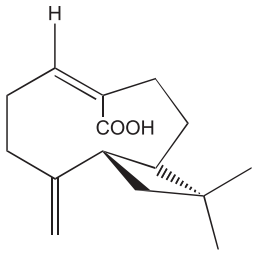
The IR spectrum of compound ***1*** showed absorption bands due to conjugated carboxylic function group (3600-2400, 1680 cm^{-1}), carbon-carbon double bonds (1640, 1470, 890 cm^{-1}), and *gem*-dimethyl groups (1370 cm^{-1}). Its ^1H NMR spectrum (Table 1) exhibited characteristic signals indicating 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].



1



2



3

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$^{\hat{}}$)	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.

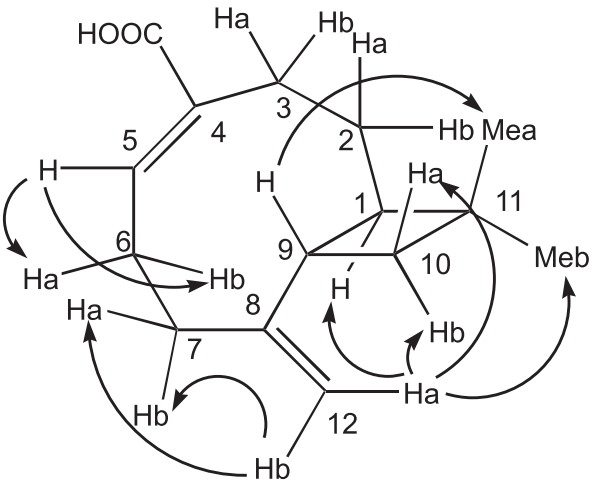


Figure 1. nOe assignments for lychnophoic acid (*1*) by NOESY experiment (ns 16, ds 4, d8 0.5 sec, TD 2K)

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].

Acknowledgements

The authors are grateful to Prof. E. Chiari, Departamento de Parasitologia, Instituto de Ciênci-

as Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil for the assays against *Trypanosoma cruzi*; T. M. S. Grandi, J. R. Stehmann and A. M. G. Anjos, Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, for the plants collection; to Prof. H. Wagner, Institut für Pharmazeutische Biologie, Universität München for the NMR and mass spectra; to LAREMAR, Laboratório de Ressonância Magnética de Alta Resolução, Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil for some NMR spectra and to CAPES, FAPEMIG and CNPq for financial support.

Recebido em: 05/08/2004
Aceito em: 19/11/2004

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.

References

[1] M.B.S. Cerqueira, J.T. Souza, R. Amado Jr., A.B.F. Peixoto. Ciência e Cultura 39 (5/6) (1987) 551-553.
[2] F. Bohlmann, J. Jakupovic. Plant Systematic Evolution 4 [S] (1990) 3-43.
[3] E. Chiari, A.B. Oliveira, D.S. Raslan, A.A.L. Mesquita, K.G. Tavares. Transactions of the Royal Society of tropical Medicine and Hygiene; 85; (1991) 372-4.
[4] E. Chiari, K.S.P. Perry, D.A. Saúde, D.S. Duarte, D.S. Raslan, M.A.D. Boaventura, , A.B. Oliveira, T.S.M. Grandi. Phytotherapy Research 10 (1996) 636-638.
[5] D.S. Duarte, D.S. Raslan, E. Chiari, A.B. Oliveira. Memorias do Instituto Oswaldo Cruz 88 [S] (1993) 240.
[6] A.B. Oliveira, D.A. Saúde, K.S.P. Perry, D.S. Duarte, D.S. Raslan, M.A.D. Boaventura, E. Chiari. Phytotherapy Research 10 (1996) 292-295.
[7] D.S. Duarte, D.A. Saúde, D.S. Raslan, M.A.D. Boaventura, K.S.P. Perry. Acta Horticulturae, 501 (2) (1999) 145-148.
[8] F. Bohlmann, C. Zdero, H. Robinson, R. M. King. Phytochemistry 19 (11) (1980) 2381-2385.
[9] P. W. Le Quesne, M. D. Menachery, M. P. Pastore, C. J. Kelley, T. F. Brennan, K. D. Onan, R. F. Raffauf, C. M. Weeks. Journal of Organic Chemistry 47 (8) (1982) 1519-21.
[10] D. Manns, R. Hartmann. Planta Medica 58 (1992) 442-444.