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Article

Pyrrolizidine Alkaloids from *Heliotropium indicum*

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Um novo alcalóide denominado Helindicina (**1**), e o conhecido licopsamina (**2**) foram isolados das raízes de *Heliotropium indicum* (Boraginaceae). As estruturas foram estabelecidas tendo como base um amplo estudo de 1D e 2D RMN (COSY, HMBC, HMQC, NOESY) e HREIMS. Este representa o primeiro relato de um alcalóide contendo um anel lacônico no gênero *Heliotropium*. Estes alcalóides mostraram moderada atividade antioxidante.

Helindicine (**1**), a new pyrrolizidine alkaloid with unusual structural features, together with the known lycopsamine (**2**), were isolated from the roots of *Heliotropium indicum* (Boraginaceae). The structures were established by a combination of 1D and 2D NMR methods (COSY, HMQC, HMBC, and NOESY) and HREIMS. This is the first report of a lactone pyrrolizidine alkaloid in the genus *Heliotropium*. Compounds **1** and **2** were assayed for antioxidant activity and showed moderate activity.

Keywords: *Heliotropium indicum*, Boraginaceae, pyrrolizidine alkaloids, antioxidant activity

Introduction

Pyrrolizidine alkaloids are considered of great pharmacological, biological and chemotaxonomic interest.[1-3] These metabolites have been isolated from a wide variety of plants, especially from genera belonging to the Boraginaceae family.[4-6] The genus *Heliotropium*, a well-known source of such alkaloids[7-9] and other minor compounds, such as flavonoids and geranyl aromatic derivatives, is constituted of about 250 species represented by herbs and shrubs, distributed throughout the terrestrial globe.[10]

In the course of a search for novel and biologically active compounds from plants the EtOH extract from the roots of *Heliotropium indicum* L., popularly known as *fedegoso*, found abundantly in the region of northeast Brazil, was investigated. This species is widely used in folk medicine in the treatment of skin disease, and as a powerful expectorant.[11] Based on a literature survey, the investigated species has been the subject of several previous studies, leading to the isolation of pyrrolizidine alkaloids.[3,12] This paper describes the isolation and structure elucidation of the new pyrrolizidine alkaloid **1**, and the known alkaloid lycopsamine (**2**).

Results and Discussion

Alkaloid **1** was isolated as a colorless oil. Its molecular formula of $C_{15}H_{23}NO_4$, was deduced by HR-EIMS (*m/z* 281.1627, M^+). The IR spectrum exhibited characteristic absorptions of hydroxyl and carbonyl groups at ν_{max} 3435 and 1726 cm^{-1}, respectively. Its EI-MS gave a molecular ion peak at *m/z* 281, and a base peak at *m/z* 207 (**1a**, $C_{11}H_{13}NO_3$), along with unusual PA fragmentation ions at *m/z* 149 (**1b**, 37%, $C_9H_{11}NO$), 135 (**1c**, 33%, C_8H_9NO), 115 (**1d**, 15%, $C_6H_{11}O_2$), 109 (**1e**, 34%, $C_7H_{11}N$), 95 (**1f**, 55%, C_6H_9N) and 81 (**1g**, 46%, C_5H_7N), compatible with the proposed structure (Figure 1).

The ^{13}C NMR spectrum displayed a total of fifteen carbon signals, while the DEPT experiment evidenced the

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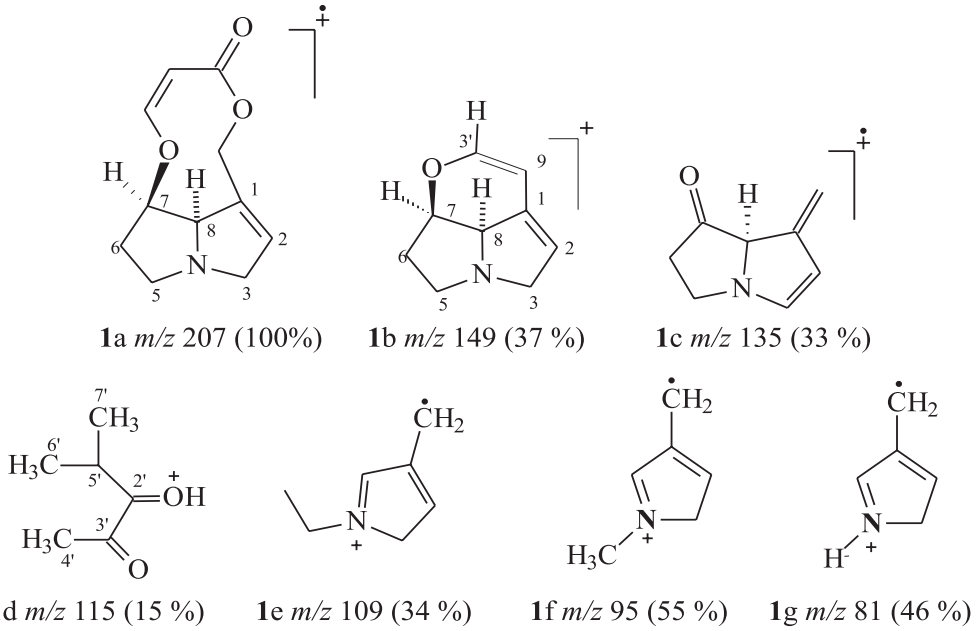

Figure 1. Fragments proposed to justify peaks observed in the mass spectrum of **1**.

presence of three methyls, four methylenes and five methines, and consequently, three quaternary carbon atoms. The ^{1}H NMR spectrum of **1** exhibited signals at δ_H 0.93 (d, *J* 7.0 Hz, H_3-7'), 0.95 (d, *J* 7.0 Hz, H_3-6'), and 2.03 (hept, *J* 7.0 Hz, H-5'), characteristic of an isopropyl group, along with an oxymethine group, showing a resonance at δ_H 4.05 (q, *J* 6.2 Hz, H-3') and an additional methyl signal at δ_H 1.17 (d, *J* 6.2 Hz, H_3-4'). These hydrogen signals were correlated with the carbon signals at δ_C 17.6 (CH_3-7'), 17.2 (CH_3-6'), 34.2 (CH-5'), 70.5 (CH-3'), and 17.3 (CH_3-4'), respectively, in the HMQC spectrum. Both H-3' and H-5' displayed ^{3}*J*-HMBC correlations with the ester carbonyl group at δ_C 175.6 (C-1'). These data are in agreement with a viridiflorate ester moiety.[13-15] A deshielded hydrogen signal at δ_H 5.94 (s, H-2) in the ^{1}H NMR spectrum, together with two olefinic carbon signals at δ_C 125.3 (CH-2) and 134.2 (C-1), suggested the presence of a trisubstituted double bond. Signals assigned to oxygenated carbon atoms were observed at δ_H 70.5 (CH-7) and 61.7 (CH_2-9), which showed correlations with the hydrogen signals at δ_H 4.63 (br s, H-7) and 4.98 (d, *J* 13.8 Hz, H-9a) and 4.80 (d, *J* 13.8 Hz, H-9b), respectively. Additionally, signals corresponding to three carbon atoms attached to nitrogen were also observed at δ_C 80.5 (CH-8), 62.5 (CH_2-3), and 55.5 (CH_2-5). These data were all consistent with a retronecine moiety.[12-14] As expected, the HMBC spectrum of **1** revealed a correlation between H-7 and the oxymethine carbon atom CH-3', as well as a correlation between H-3' and CH-7. The NOESY correlation between the oxymethine hydrogen H-7 and the methyl hydrogens H_3-4', both in the *a*-position, confirmed this deduction. The relative stereochemistry of **1** was established based on coupling constant values and the NOESY experiment (Figure 2).

It is worth noting that helindicine (**1**) is an unusual 9-membered lactone pyrrolizidine alkaloid, which is formed by an ether linkage between C-3' of the viridiflorate ester side chain and the C-7 of the retronecine moiety. Moreover, this is the first example

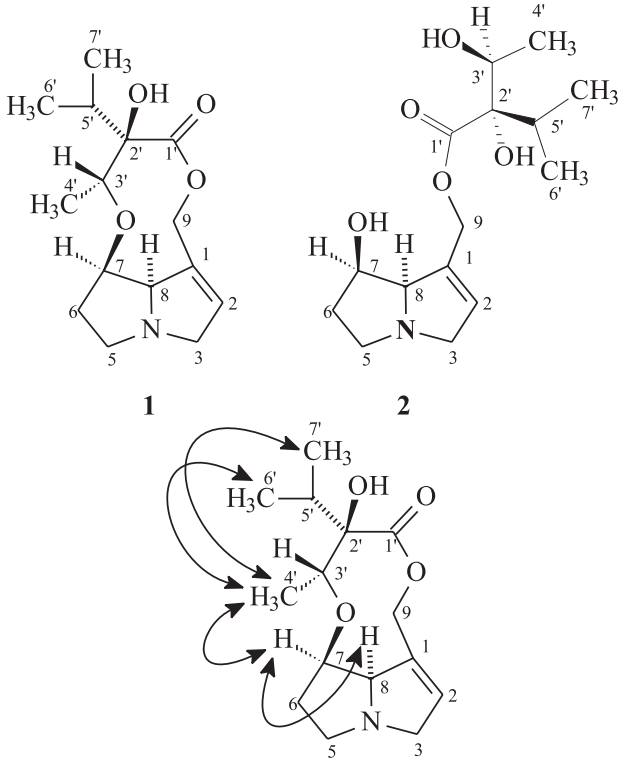

Figure 2. Important NOESY correlations of **1**.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data for helindicine (***1***) and lycopsamine (***2***) in CD_3OD as solvent

	1				**2**	
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H
1	134.2		H-2, H-8		133.2	
2	125.3	5.94 (br s)	2H-3, H-8		129.7	5.87 (br s)
3	62.5	4.40 (br d, *J* 15.0)	H-2	H-5b	62.9	3.96
		3.92 (br d, *J* 15.0)				3.45
5	55.5	3.97 (m)		2H-3	54.1	3.36 (m)
		3.34 (m)				2.78 (m)
6	36.7	2.15 (2H, m)	H-5b		36.5	1.98 (2H, m)
7	70.5	4.63 (br s)	2H-6	2H-5, H-3'	69.6	4.33 (br s)
8	80.5	4.90 (br *s*)		H-2, 2H-6	78.8	4.29 (br s)
9	61.7	4.98 (d, *J* 13.8)		H-2	62.8	5.09 (d, *J* 12.7)
		4.80 (d, *J* 13.8)				4.58 (d, *J* 12.7)
1'	175.6			2H-9, H-3',H-5'	175.7	
2'	84.5		H-5'	3H-4', 3H-6', 3H-7'	83.3	
3'	70.5	4.05 (q, *J* 6.2)	3H-4'	H-7	71.4	4.04 (q, *J* 6.4)
4'	17.3	1.17 (d, *J* 6.2)			17.5	1.16 (d, *J* 6.4)
5'	34.2	2.03 (hept. *J* 7.0)	3H-6', 3H-7'		32.9	2.78 (m)
6'	17.2	0.95 (d, *J* 7.0)	H-5'		17.1	0.93 (d, *J* 7.0)
7'	17.6	0.93 (d, *J* 7.0)	H-5'		17.7	0.91 (d, *J* 7.0)

[a] All assignments were based on DEPT, COSY, HMQC, HMBC and NOESY experiments. Coupling constants (*J*) in Hz for hydrogen atoms were obtained of the 1D ^{1}H NMR spectra. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC and ^{1}H-^{1}H-COSY spectra.

of a lactone pyrrolizidine alkaloid isolated from the genus *Heliotropium*.

The more polar isolated alkaloid was identified as lycopsamine (**2**), by comparison of its physical and spectral properties with literature data.[7,15]

The results of an extensive application of 1D (^{1}H NMR, proton broad-band decoupled and DEPT-^{13}C NMR) and 2D (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HMQC and HMBC) spectral techniques were also used to establish the complete ^{1}H and ^{13}C resonance assignments of these two pyrrolizidine alkaloids (**1** and **2**).

Structural examination of the these compounds in view of using biosynthetic arguments and application of a biosynthetic retroanalysis led us to suggest biogenetic route and, consequently, the alkaloid lycopsamine (**2**) may be postulated as precursor of helindicine (**1**) through a dehydration reaction. The antioxidant activities of **1** and **2**, together with standard radical scavenging trolox and BHT were tested and compared (Table 2). As can be seen, **1** and **2** showed moderate free radical scavenging ability. The higher level of lycopsamine (**2**) as compared to helindicine (**1**) is probably due to the free hydroxyl group.

Alkaloids **1** and **2** were also tested for binding to estrogen receptor α and β, and alkaline phosphatase induction in Ishikawa cells. Neither alkaloid showed activity (>50%) in these assays.

Experimental

General

The optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1000 FT-IR spectrometer. Mass spectra data (EI-MS and HR-EIMS) were acquired on a Shimadzu QP5050A and a JEOL CGMate II instrument, through direct probe and operating at 70 eV. NMR

Table 2. The DPPH free radical scavenging activity of alkaloids **1** and **2**. The free radical scavenging effect was measured by the absorbance radical at 520 nm in a reaction containing the test sample and 60 μmol L^{-1} DPPH

Treatment	Concentration/(mg mL^{-1})					
	1.0		0.5		0.25	
	Activity	%	Activity	%	Activity	%
1	0.1852	36.00	0.1925	34.00	0.1941	33.90
2	0.1700	42.10	0.1727	41.02	0.1743	40.70
Trolox	0.0110	95.60	0.0110	95.60	0.0110	95.60
BHT	0.0824	64.00	0.0871	62.00	0.1172	48.60
Control	0.2285	00.00	0.2285	00.00	0.2285	00.00

experiments were performed on a Bruker DRX-500 [^{1}H (500 MHz) and ^{13}C (125 MHz)] and Varian UM-400 [^{1}H (400 MHz) and ^{13}C (100 MHz)] spectrometers. Silica gel 60 (70-230 mesh, Merck) and Sephadex LH-20 (Pharmacie) was used for column chromatography. TLC was performed on silica gel sheets over polyethylene (Kieselgel 60 F_{254}, 0.20 mm, Merck). Compounds were detected by spraying with Dragendorff's reagent and reaction with iodine vapors.

Plant material

The roots of *H. indicum* were harvested from Pentecoste County, State of Ceará – Brazil, in January 2003. The plant material was authenticated by Professor Edson P. Nunes, and a voucher specimen (# 31.610) has been deposited at the Herbário Prisco Bezerra (EAC) of the Departamento de Biologia, Universidade Federal do Ceará.

Extraction and isolation

Dried and powdered roots of *H. indicum* (1200 g) were exhaustively extracted with EtOH at room temperature (x 3). The combined extracts were evaporated in vacuum to yield a crude extract (90.0 g), which was processed in the usual way as for alkaloid extraction.[16] The alkaloid fraction (10.0 g) was separated on a silica gel column, using *n*-hexane, $CHCl_3$, EtOAc, and MeOH successively as eluents. The MeOH fraction (3.5 g) was initially suspended in acetone, and then MeOH. The acetone-soluble fraction (1.1 g) was chromatographed on Si gel using a stepwise gradient solvent system of hexane-EtOAc (1:1), EtOAc, EtOAc-acetone (1:1), acetone, acetone-MeOH (1:1), and MeOH to obtain 10 fractions, each of 50 mL. The fractions were monitored by TLC and pooled into three fractions. Fraction 3 (819 mg) was rechromatographed on Si gel using EtOAc with increasing amounts of MeOH (10 – 80%) to yield 4 subfractions. Fraction 1 (70 mg) was chromatographed on a column of Sephadex LH-20 using MeOH as eluent to yield **1** (64 mg). The MeOH-soluble fraction (589 mg) was chromatographed on a Si gel column and eluted with a stepwise gradient solvent system of EtOAc-acetone (8:2 to 2:8) and acetone-MeOH (8:2 to 8:2) to obtain 36 fractions, which were monitored by TLC and pooled to 10 fractions. The fraction 5, obtained with EtOAc-acetone (7:3), yielded **2** (48.5 mg).

Identification of the isolated alkaloids

*Helindicine (**1**).* Colorless resin; $[\alpha]_D^{20}$ -0.64° (*c* 0.05, CH_3OH); IR ($CHCl_3$) ν_{max}/cm^{-1}: 3435, 1726, 1636, 1244, 1030; HR-EIMS (M^+, *m/z* 281.2); EI-MS *m/z* (rel. int.): 281 $[M]^{+\cdot}$ (35), 207 (100), 191 (15), 149 (37), 135 (33), 109 (34), 97 (73), 95 (55), 83 (57), 81 (46); ^{1}H and ^{13}C NMR (CD_3OD) (500 and 125 MHz, respectively) data, see Table 1.

*Lycopsamine (**2**).* Colorless resin; $[\alpha]_D^{20}$ +0.12° (*c* 0.05, $CHCl_3$); IR ($CHCl_3$) ν_{max}/cm^{-1}: 3411, 1732, 1559, 1236, 1022; HR-EIMS (M^+, *m/z* 299.2); EIMS: *m/z* 299 $[M^+]$ (1.6), 254 (1.5), 156 (13), 138 (100), 120 (15), 108 (5.0), 93 (47), 80 (26); ^{1}H and ^{13}C NMR (CD_3OD) (500 and 125 MHz, respectively) data, see Table 1.

DPPH assay

The antioxidant activities of compounds **1** and **2** were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. One milliliter of a 60 μmol L^{-1} DPPH ethanol solution was added to sample solutions at three different concentrations and allowed to react at room temperature. After 30 min, the absorbance values were measured at 520 nm using a spectrophotometer and converted into the percentage antioxidant activity using a known procedure.[17,18] Blank experiment were also carried out to determine the absorbance of DPPH before interacting with the compounds. Trolox and BHT were used as positive control compounds.

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