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Article

A New Indole Alkaloid Isolated from *Tabernaemontana hystrix* Steud (Apocynaceae)

Cecilia Silva Monnerat, Jucimar Jorgeane de Souza, Leda Mathias, Raimundo Braz-Filho and Ivo José C. Vieira*

Setor de Química de Produtos Naturais, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 28013-602 Campos dos Goytacazes - RJ, Brazil

Um novo alcalóide, denominado histrixnina (**1**), e cinco alcalóides indólicos conhecidos, ibogamina (**2**), olivacina (**3**) e affinina (**4**), affinisina (**5**) e N_b-metilaffinisina (**6**), foram isolados do extrato metanólico das cascas das raízes de *Tabernaemontana hystrix*. Os triterpenos conhecidos 3-*O*-acetil-α-amirina, 3-*O*-acetil-β-amirina, 3-*O*-acetil-lupeol foram também identificados. As estruturas dos compostos foram elucidadas com base na análise de dados espectroscópicos.

A new alkaloid, named hystrixnine (**1**), and five known indole alkaloids, ibogamine (**2**), olivacine (**3**), affinine (**4**), affinisine (**5**) and N_b-methylaffinisine (**6**), were isolated from the root bark of *Tabernaemontana hystrix*. The known triterpenes α-amyrin acetate, β-amyrin acetate and lupeol acetate were also identified. The structures of the compounds were elucidated based on spectroscopic studies.

Keywords*: Tabernaemontana hystrix*, Apocynaceae, indole alkaloids, triterpenes

Introduction

Indole alkaloids exhibit numerous biological activities (such as anti-tumor, anti-microbial, anti-hypertensive and central nervous system stimulant).[1] They can be found in plants of the Apocynaceae, Rubiaceae, and Loganiaceae families.[1,2]

Among the Apocynaceae, the genus *Tabernaemontana* is especially rich in indole alkaloids. They are useful chemical markers of the genus, and also have a great value for the classification of the individual species within the genus.[3] The classification of individual species only on the basis of morphological characters has been difficult, leading to numerous synonyms.[4]

The species *Tabernaemontana hystrix* Steud. should have the homotypic synonym *Tabernaemontana echinata* Vell. and *Peschiera hystrix* (Steud.) A. DC., and the heterotypic synonyms *T. collina* Gardn. in Hooker, *T. fuchsiifolia* (A. DC.), *Peschiera fuchsiifolia* (A. DC.) Miers, *T. gaudichaudii* A. DC., *T. lundii* A. DC., *Peschiera lundii* (A. DC.) Miers, *T. gracilis* Muell., *T. bracteolaris* Muell., *Peschiera granulosa* Miers and *Peschiera solandri* Miers.[4] In fact, previous phytochemical studies have been published under the name *Peschiera fuchsiifolia* (A. DC.) Miers.[5,6]

* e-mail: curcino@uenf.br

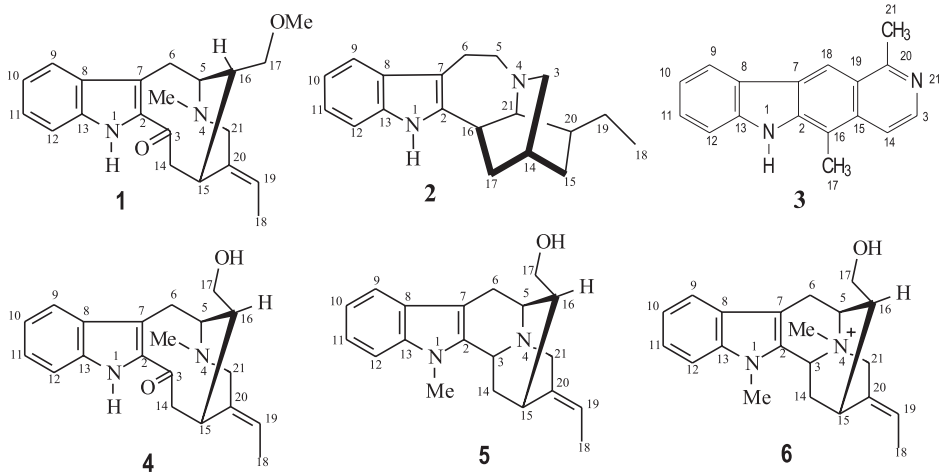
As part of our continuing interest in the phytochemical investigation of *Tabernaemontana* species occurring in Brazil,[7-9] we decided to study *T. hystrix,* a native species of the Atlantic forest in Southeastern Brazil, popularly known as "esperta".

In the present work, we report the phytochemical analysis of the crude methanolic extract of *T. hystrix*, which allowed to characterize the presence of six indole alkaloids (**1** to **6**), including the new one named hystrixnine (**1**), and three triterpenoids. The structures were established by spectrometric techniques, mainly EIMS and 1D and 2D NMR, including comparative analysis with literature values.

Results and Discussion

Chromatographic purification of *T. hystrix* root bark methanol extract yielded triterpenes common in plants, including other *Tabernaemontana* species.[10] The triterpene acetates were obtained as a mixture of α-amyrin acetate, β-amyrin acetate and lupeol acetate. They were identified by ^{1}H and ^{13}C NMR spectral data compared with literature values.[11]

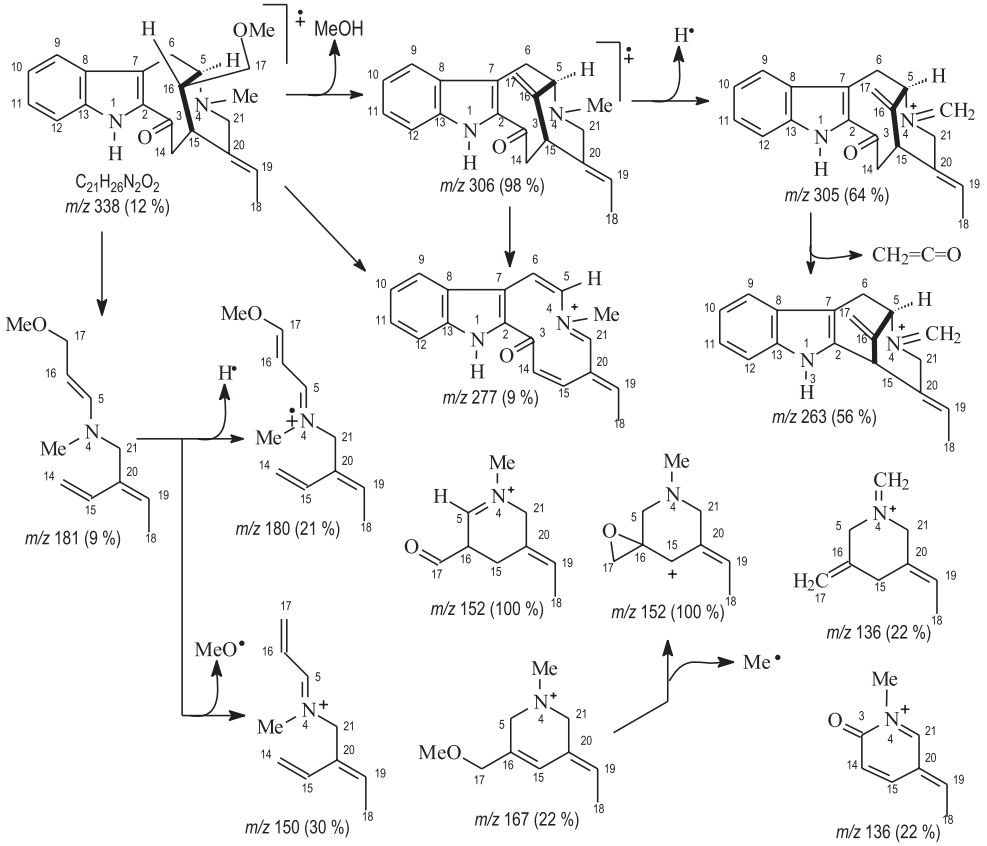
The known indole alkaloids, ibogamine (**2**),[12,13] olivacine (**3**),[14,15] affinine (**4**),[5,16,17] affinisine (**5**)[5,6,17] and N_b-methylaffinisine (**6**)[6] were identified on the basis of ^{1}H and ^{13}C NMR spectral data, including homonuclear ^{1}H-^{1}H-COSY and heteronuclear ^{1}H-^{13}C 2D shift-correlated



NMR experiments, which were also used to complete and unambiguous ^{1}H and ^{13}C chemical shift assignments.[18]

The UV spectrum of hystrixnine (**1**) showed absorptions at λ_{max} 223 and 282 nm (ε 42566 and 6287, respectively) typical of an substituted indole chromophore,[8] while the IR spectrum revealed bands at ν_{max} 3360 (N-H), 1736 (conjugated carbonyl ketone group stretching), 2930-2830 (C-H stretching) and 1616, 1591 and 743 cm^{-1} (C-H bending of benzene ring).[8] The EIMS showed a molecular peak at *m/z* 338 daltons ($[M]^{+\cdot}$) which together with ^{1}H and ^{13}C NMR spectral data (Table 1) allowed to deduce the molecular formula $C_{21}H_{26}N_2O_2$ (ten degrees of unsaturation) compatible with corynanthean skeleton.[8] The principal peaks observed in the EIMS spectrum are in agreement with proposed fragmentation mechanisms summarized in Scheme 1.

Carbon-13 NMR experiments ({^{1}H} and APT) revealed the presence of three methyl groups, four methylenes (sp^3), eight methines (three sp^3 and five sp^2) and six (sp^2) quaternary carbon atoms. The ^{1}H-^{1}H-COSY, HMQC and



Scheme 1. Fragments proposed to justify the main peaks observed in the ESMS of **1**.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR for hystrixnine (**1**), including results obtained by heteronuclear 2D shift-correlated HMQC ($^{1}J_{CH}$) and HMBC ($^{n}J_{CH}$, n=2 and 3) and comparison with **4**, in $CDCl_3$ as solvent. Chemical shifts (δ, ppm) and coupling constants (J, Hz, in parenthesis)*

	1				**4**	
	^{1}H-^{13}C-HMQC-$^{1}J_{CH}$		^{1}H-^{13}C-HMBC-$^{n}J_{CH}$			
C	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$	δ_C	δ_H
2	135.43	-		2H-6; H-14a	136.80	-
3	191.50	-	H-14b	H-15	194.00	-
7	120.36	-	H-6	HN-1; H-9	122.00	-
8	128.30	-		H-6; H-10; H-12	129.60	-
13	136.41	-		H-9; H-11	138.60	-
20	134.71	-	2H-21	3H-18	136.32	-
CH						
5	56.93	3.31 (br d, 8.4)		Me-4; H-15; 2H-17; H-21b	56.97	3.06 (br t, 8.3)
9	120.48	7.70 (d, 8.1)		H-11	121.64	7.68 (br d, 8.1)
10	120.64	7.16 (dd, 8.4, 8.1)		H-12	121.11	7.09 (ddd, 8.3, 8.1, 1.1)
11	126.81	7.36 (dd, 8.4, 8.4)		H-9	127.44	7.28 (ddd, 8.3, 8.3, 1.1)
12	112.19	7.49 (br d, 8.4)		H-10	113.40	7.40 (br d, 8.3)
15	31.60	3.07 (br t, 8.8)	2H-14	2H-17; H-19; H-21b	31.29	3.04 (br t, 8.3)
16	38.02	1.97 (m)	H-5; H-15	2H-6; 2H-14	41.27	1.89 (t, 6.7)
19	121.31	5.49 (br q, 7.0)	3H-18	2H-21	122.50	5.49 (br q, 6.7)
CH_2						
6	19.34	3.54 (m)	H-5		20.60	3.34 (m)
		3.48 (m)				3.29 (m)
14	43.50	3.33 (m)	H-15		44.30	3.25 (dd, 12.6, 9.7)
		2.67 (dd, 12.8, 7.7)				2.45 (dd, 12.6, 6.7)
17	67.44	3.62 (dd, 8.0, 2.2)		H-5; H-15	65.61	3.41 (dd, 6.2, 1.1)
		3.59 (dd, 8.0, 2.2)				
21	52.04	3.70 (br d, 13.9)		MeN-4; H-5; H-15; H-19	53.27	3.50 (br d, 13.7)
		3.04 (d, 13.9)				2.83 (d, 13.7)
CH_3						
18	12.10	1.70 (dd, 7.0, 2.2)	H-19		12.20	1.65 (dd, 6.7, 1.9)
MeN	41.81	2.57 (s)			42.30	2.41 (s)
MeO	50.74	3.47 (s)			-	-
HN-1	-	9.32 (br s)			-	9.15 (br s)

*Number of hydrogens bound to carbon atoms deduced by comparative analysis of ^{1}H- and APT-^{13}C NMR spectra. Chemical shifts and coupling constants (J) obtained of 1D ^{1}H NMR spectrum. Superimposed ^{1}H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC and ^{1}H-^{1}H-COSY spectra. All ^{1}H and ^{13}C chemical shift assignments of **1** were also based on homonuclear ^{1}H-^{1}H-COSY and heteronuclear 2D shift-correlated HMQC ($^{1}J_{CH}$) and HMBC ($^{n}J_{CH}$, n=2 and 3) NMR.

HMBC experiments established *geminal* and vicinal hydrogen interactions as well as direct ($^{1}J_{CH}$) and two and three bond correlations between carbon and hydrogen atoms in the structure (Table 1). These data revealed that **1** is closely related to affinine (**4**), differing by the presence of methoxyl group linkage at C-17. The presence of the indole nucleus was clearly indicated by the ^{1}H and ^{13}C aromatic signals (Table 1). Typically the ^{1}H NMR revealed two singlet signals at δ_H 3.47 (MeO-17) and 2.57 (MeN-4) and double doublet signal at δ_H 1.70 (J= 7.0 and 2.2 Hz, 3H-18 linkage at sp^2 CH-19) corresponding to methyl groups. Through analysis of the HMBC spectrum these signals were assigned by corresponding cross-peaks, due to heteronuclear spin-spin coupling via three ($^{3}J_{CH}$) bonds, two methyl groups linked to the allylic Me-18 and N-4 (aliphatic N_b), respectively: *i*) Me-18 (δ_H 1.70) with C-20 (δ_C 134.71); *ii*) and MeN-4 (δ_H 2.57) with both CH-5 (δ_C 56.93) and CH_2-21 (δ_C 52.04). The ketone group localized at position C-3 was confirmed by correlations with H-14b [δ_H 3.33 ($^{2}J_{CH}$)] and H-15[(δ_H 3.07 ($^{3}J_{CH}$)]. The presence of methoxyl group was confirmed by ^{1}H NMR and ^{13}C NMR spectra by presence of the signals at δ_H 3.47 (s) and δ_C 50.74 (Table 1). The complete analysis of this HMBC spectrum in combination with additional NMR spectral data also allowed the identification of a skeleton as that of the indole alkaloid affinine (**4**)[5,16,17] and the total ^{1}H and ^{13}C chemical shift assignments, as summarized in Table 1. Thus, the new alkaloid corynanthean skeleton isolated from *Tabernaemontana hystrix* was characterized as **1**, named hystrixnine.

In accordance with the revision published by Leeuwenberg,[4] the alkaloid series isolated in this study from *T. hystrix* are closely related to those previously reported from *Peschiera fuchsiifolia*: decarbomethoxy-

voamine, demethylvoacamine, voacamidine, perivine, 16-epiaffinine, voacangine hydroxyindolenine, fuchsiaefoline, 12-methoxy-N_b-methylvoachalotine and 12-methoxy-N_b-methylvoachalotine ethyl ester (reported by Braga and co-workers).[5,6] The similarity of the alkaloids reported in this work in comparison with those from two other Brazilian *Tabernaemontana* species is remarkable. From *T. solanifolia* were reported the alkaloids: isovoacangine, isovoacristine, coronaridine, voacangine, voacangine hydroxyindolenine, heyneanine, voacamine, voachalotine and 12-methoxy-Nb-methylvoachalotine [reported under the name *Peschiera campestris* (Rizz.) Rizz. by Gower *et al.*][19] and *P. laeta* were described the alkaloids: coronaridine, voacangine, isovoacangine, 19-(*S*)-heyneanine, isovoacristine, 3-oxoisovoacangine, ibogaine, iboxygaine, tabersonine, apparicine, vobasine, N_b-methylvoachalotine, voacamine, conodurine and tabernamine [reported under the name *T. laeta* (Mart.) by Medeiros and co-workers].[7-9] This similarity might point to a close taxonomic relationship of these recognized species.

Experimental

General

^{1}H NMR and ^{13}C NMR: At Jeol Eclipse spectrometer operating at 400 MHz and 100 MHz, respectively, in $CDCl_3$, using the residual solvent signals as internal standard (Table 1).

Plant materials

The root bark of *Tabernaemontana hystrix* Steud. was collected in March 2002 at Varre e Sai, Rio de Janeiro State, Brazil, and identified by Dr. A. J. M. Leeuwenberg of the Agricultural University of Wageningen, The Netherlands. A voucher specimen (WAG) is deposited at the herbarium of the Agricultural University of Wageningen, Netherlands.

Extraction and isolation

Dried and powdered root bark (0.92 kg) from *T. hystrix* Steud. was extracted at room temperature using methanol, furnishing after solvent evaporation, crude methanol extracts (40.0 g).

23.0 g of the methanol extract was chromatographed on a Si gel column and eluted with a gradient of MeOH in CH_2Cl_2, yielding 11 fractions. The fractions 1-3 (460 mg) was recrystallizated from hexane to furnish a mixture of the three triterpenes (180 mg) α-amyrin acetate, β-amyrin acetate and lupeol acetate; fraction 5 (940 mg) furnished **2** (58 mg); fraction 8 (1.58 g) was rechromatographed on a Si gel column using a gradient of MeOH in CH_2Cl_2 affording **5** (73 mg); fraction 9 (1.36 g) was rechromatographed in the same way, yielding the alkaloids **3** (73 mg), **4** (26 mg) and **6** (11 mg). 2.6 g of fraction 10 was rechromatographed on a Si gel column using a gradient of MeOH in CH_2Cl_2 furnishing 06 fractions, of which, fraction 4 (54 mg) furnished the alkaloid **1** (7.9 mg) after rechromatography with a mixture of MeOH in CH_2Cl_2.

The four alkaloids **2-6**, as well as three triterpenes were identified by the analysis of ^{1}H and ^{13}C NMR and comparison with literature values.[11-17]

*hystrixnine (**1**)*. Amorphous solid; $[\alpha]^{25}_D$ - 100° (MeOH, *c* 0.66); IR (KBr) ν_{max}/cm^{-1} 1736 (C=O), 1616, 1591, 743 (aromatic ring); UV λ_{max}/nm (CH_3OH) 223 (ε 42566), 282 (ε 6287). EIMS: Scheme 1; ^{1}H NMR and ^{13}C NMR: Table 1.

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