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

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Um novo alcalóide, denominado histrixnine (1), e cinco alcalóides indólicos conhecidos, ibogamine (2), olivacine (3) e affinina (4), affinisina (5) e N₅-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 N₅-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). They can be found in plants of the Apocynaceae, Rubiaceae, and Loganiaceae families.

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. The classification of individual species only on the basis of morphological characters has been difficult, leading to numerous synonyms.

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. fuchsifolia* (A. DC.), *Peschiera fuchsifolia* (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. In fact, previous phytochemical studies have been published under the name *Peschiera fuchsifolia* (A. DC.) Miers.

As part of our continuing interest in the phytochemical investigation of *Tabernaemontana* species occurring in Brazil, 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 spectroscopic 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. The triterpene acetates were obtained as a mixture of α-amyrin acetate, β-amyrin acetate and lupeol acetate. They were identified by ¹H and ¹³C NMR spectral data compared with literature values.

The known indole alkaloids, ibogamine (2), olivacine (3), affinine (4), affinisine (5) and N₅-methylaffinisine (6) were identified on the basis of ¹H and ¹³C NMR spectral data, including homonuclear ¹H-¹H-COSY and heteronuclear ¹H-¹³C 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 \(\lambda_{\text{max}}\) 223 and 282 nm (\(\varepsilon\) 42566 and 6287, respectively) typical of an substituted indole chromophore,\(^8\) while the IR spectrum revealed bands at \(\nu_{\text{max}}\) 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]+) which together with \(^1\)H and \(^{13}\)C NMR spectral data (Table 1) allowed to deduce the molecular formula \(\text{C}_{21}\text{H}_{26}\text{N}_{2}\text{O}_{2}\) (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](image_url)
HMBC experiments established *geminal* and vicinal hydrogen interactions as well as direct (\(J_{	ext{CH}}\)) 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 1H and 13C aromatic signals (Table 1). Typically the 1H NMR revealed two singlet signals at \(\delta_H 3.47\) (MeO-17) and 2.57 (MeN-4) and double doublet signal at \(\delta_H 1.70\) (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 (\(3J_{	ext{CH}}\)) bonds, two methyl groups linked to the allylic Me-18 and N-4 (aliphatic N\(_c\)), respectively: i) Me-18 (\(\delta_H 1.70\)) with C-20 (\(\delta_C 134.71\)); ii) and MeN-4 (\(\delta_H 2.57\)) with both CH-5 (\(\delta_C 56.93\)) and CH\(_2\)-21 (\(\delta_C 52.04\)). The ketone group localized at position C-3 was confirmed by correlations with H-14b \([\delta_H 3.33\) (\(2J_{	ext{CH}}\))] and H-15\([\delta_H 3.07\) (\(3J_{	ext{CH}}\))]. The presence of methoxyl group was confirmed by 1H NMR and 13C NMR spectra by presence of the signals at \(\delta_H 3.47\) (s) and \(\delta_C 50.74\) (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 1H and 13C 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 fuchsifolia*: decarbomethoxy-

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**Table 1.** H (400 MHz) and 13C (100 MHz) NMR for hystrixnine (1), including results obtained by heteronuclear 2D shift-correlated HMQC (\(J_{	ext{CH}}\)) and HMBC (\(J_{	ext{CH}}\) n=2 and 3) and comparison with 4, in CDCl\(_3\) as solvent. Chemical shifts (\(\delta\), ppm) and coupling constants (\(J\), Hz, in parenthesis)*

|      | 1H-13C-HMQC-\(J_{	ext{CH}}\) | 1H-13C-HMBC-\(J_{	ext{CH}}\) |
|------|-----------------------------|-----------------------------|
| C    | \(\delta_C\) | \(\delta_H\) | \(J_{	ext{CH}}\) | \(\delta_C\) | \(\delta_H\) |
| 2    | 135.43 | - | 2H-6; H-14a | 136.80 | - |
| 3    | 191.50 | - | H-14b | 194.00 | - |
| 7    | 120.36 | - | H-6 | 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 | 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 (d, 8.1) | H-12 | 121.11 | 7.09 (dd, 8.3, 8.1, 1.1) |
|     | 11 | 126.81 | 7.36 (d, 8.4, 8.4) | H-9 | 127.44 | 7.28 (dd, 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-19; H-21b | 31.29 | 3.04 (br t, 8.3) |
|     | 16 | 38.02 | 1.97 (m) | H-5; H-15 | 41.27 | 1.89 (t, 6.7) |
|     | 19 | 121.31 | 5.49 (br q, 7.0) | 3H-18 | 122.50 | 5.49 (br q, 6.7) |
| CH\(_2\) | 6 | 19.34 | 3.54 (m) | H-5 | 20.60 | 3.34 (m) |
|      | 14 | 43.50 | 3.33 (m) | H-15 | 44.30 | 3.25 (dd, 12.6, 9.7) |
|      | 17 | 67.44 | 3.62 (dd, 8.0, 2.2) | H-5; H-15 | 65.61 | 3.41 (dd, 12.6, 6.7) |
|      | 21 | 52.04 | 3.70 (br d, 13.9) | 3.04 (d, 13.9) | MeN-4; H-5; H-15; H-19 | 53.27 | 3.50 (br 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 1H- and APT-13C NMR spectra. Chemical shifts and coupling constants (\(J\)) obtained of 1D 1H NMR spectrum. Superimposed 1H signals are described without multiplicity and chemical shifts deduced by HMQC, HMBC and 1H-1H-COSY spectra. All 1H and 13C chemical shift assignments of 1 were also based on homonuclear 1H-1H-COSY and heteronuclear 2D shift-correlated HMQC (\(J_{	ext{CH}}\)) and HMBC (\(J_{	ext{CH}}\) n=2 and 3) NMR.*
voacamine, demethylvoacamine, voacamidine, perivine, 16-epiaffinine, voacangine hydroxyindolenine, fuchsiacoline, 12-methoxy-N_b-methylvoacalotine and 12-methoxy-N_b-methylvoacalotine ethyl ester (reported by Braga and co-workers). 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: isovoa- 

cingle, isovoaacristine, coronaridine, voacangine, voacangine hydroxyindolenine, heyneanine, voacamine, voachalotine and 12-methoxy-Nb-methylvoachalotine [reported under the name Peschiera campestris (Rizz.) Rizz. by Gower et al.] and *P. laeta* were described the alkaloids: coronaridine, voacangine, isovoaacangine, 19-(S)-heyneanine, isovoaacrinine, 3-oxoisovoacangine, ibogaine, iboxygaine, tabersonine, apparicine, vobasine, N_b-methylvoacalotine, voacamine, conodurine and tabernamine [reported under the name *T. laeta* (Mart.) by Medeiros and co-workers]. This similarity might point to a close taxonomic relationship of these recognized species.

**Experimental**

**General**

^1H NMR and ^13C 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 recrystallized 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 ^1H and ^13C NMR and comparison with literature values.

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