

Article

A New Natural Quaternary Indole Alkaloid Isolated from *Tabernaemontana laeta* Mart. (Apocynaceae)

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Um novo alcalóide quaternário natural, N_b-metilvoachalotina (**1**), três alcalóides indólicos diméricos, conodurina (**2**), voacamina (**3**) e tabernamina (**4**), e os três alcalóides monoméricos 19S-heyneanina (**5**), coronaridina (**6**) e voacangina (**7**) foram isolados da casca das raízes de *Tabernaemontana laeta*. Os triterpenos conhecidos 3-O-acetil- α -amirina, 3-O-acetil- β -amirina, 3-O-acetil-lupeol e 3-O-acetil-taraxasterol e os fitoesteróides β -sitosterol e seu derivado 3-O- β -D-glicopiranosídeo foram também identificados. As estruturas dos compostos foram elucidadas com base na análise de dados espectroscópicos.

A new natural quaternary alkaloid, N_b-methylvoachalotine (**1**), was obtained from the root bark of *Tabernaemontana laeta* together with three dimeric indole alkaloids, conodurine (**2**), voacamine (**3**) and tabernamine (**4**), and the monomeric indole alkaloids 19S-heyneanine (**5**), coronaridine (**6**) and voacangine (**7**). The known triterpenes α -amyirin acetate, β -amyirin acetate, lupeol acetate and taraxasterol acetate and the phytosterol β -sitosterol and its 3-O- β -D-glucoside were also identified. The structures of the compounds were elucidated based on spectroscopic studies.

Keywords: *Tabernaemontana laeta*, Apocynaceae, indole-alkaloids, triterpenes

Introduction

Indole alkaloids exhibit numerous biological activities (such as anti-tumor, anti-microbial, anti-hypertensive and central nervous system stimulant¹). 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 genus³. The classification of individual species only on the basis of morphological characters has been difficult, leading to numerous synonyms⁴. The species *Tabernaemontana laeta* Mart. has the homotypic synonym *Peschiera laeta* (Mart.) Miers, and the heterotypic synonyms *T. laeta* var. *pubiflora* Muell. Arg., *T. laeta* var. *minor* Muell. Arg., *T.*

laeta var. *densa* Muell. Arg., *T. spixiana* Mart. ex Muell. Arg. and *T. breviflora* Muell. Arg.⁴. Some previous phytochemical studies have been published under the name *Peschiera laeta* Mart.^{5,6}.

As part of our continuing interest in the phytochemical investigation of *Tabernaemontana* species occurring in Brazil, we decided to study *T. laeta*, a native species of the Atlantic forest in Southeastern Brazil. Popularly known as “esperta” or “guarana”, this species is generally considered poisonous. In a recent paper, we reported the complete ¹H and ¹³C chemical shift assignments of the dimeric indole alkaloids conodurine (**2**) and voacamine (**3**); both isolated from its methanolic extracts⁷.

In the present study, we report the phytochemical analysis of the crude extracts of *T. laeta*, including the isolation of the new alkaloid N_b-methylvoachalotine (**1**).

Results and Discussion

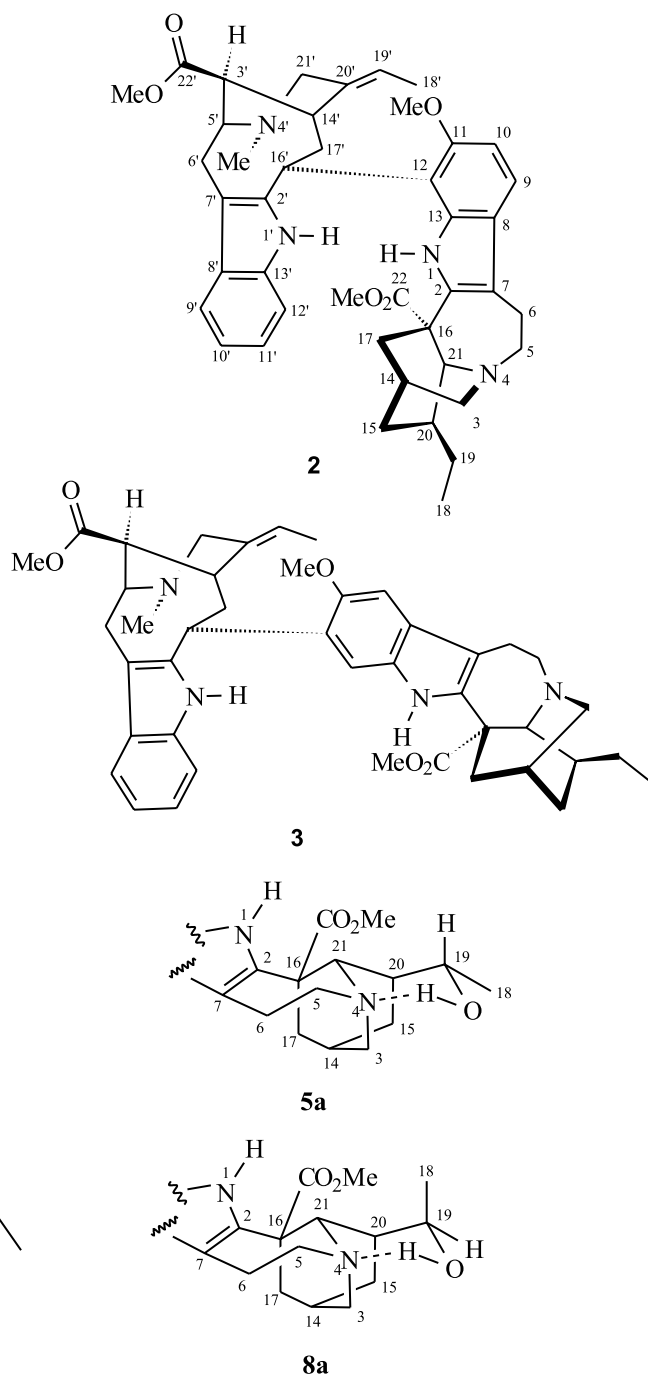
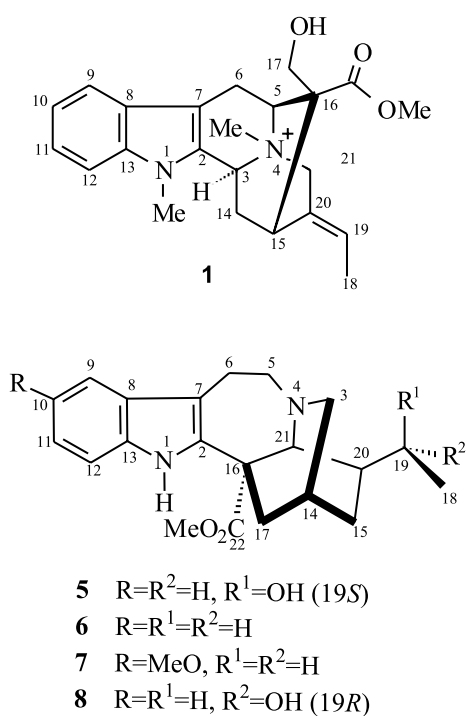
Chromatographic purification of *T. laeta* root bark hexane and methanol extracts yielded triterpenes and

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phytosterols common in plants, including other *Tabernaemontana* species⁸. The triterpene acetates were obtained as a mixture of α -amyrin acetate, β -amyrin acetate, lupeol acetate and taraxasterol acetate. They were identified by comparison of the ^1H and ^{13}C NMR spectral data with literature values⁹. The phytosterol β -sitosterol, isolated from the hexane extract, and its 3-*O*- β -D-glucopyranosyl derivative, isolated from the methanol extract, were also identified by comparison with published ^1H and ^{13}C NMR spectral data, including the data of the peracetyl derivative of 3-*O*- β -D-glucopyranosyl- β -sitosterol¹⁰.

In addition to the dimeric alkaloids conodurine (**2**) and voacamine (**3**), previously reported⁷, other structurally related alkaloids were obtained during a detailed phytochemical analysis of the methanol extract. The known indole alkaloids tabernamine (**4**)¹¹, 19*S*-heyneanine (**5**, *vide infra*), coronaridine (**6**)¹² and voacangine (**7**)¹³ were identified on the basis of ^1H and ^{13}C NMR spectral data, including homonuclear ^1H - ^1H -COSY and heteronuclear ^1H - ^{13}C 2D shift-correlated NMR experiments¹⁴.

The 19*S* configuration shown in **5** was clearly established by comparison of the NMR chemical shifts



obtained with those reported for heyneanine (**5**) and its epimer, (19*R*)-epiheyneanine (**8**). Due to hydrogen bonding between the hydroxyl group at C-19 and the nearby nitrogen the 1-hydroxyethyl moiety has a rigid conformation (see partial structures **5a** and **8a**), leading to considerable chemical shift differences. In the ¹H NMR spectrum especially the chemical shifts of the 3H-18 and H-19 hydrogens show large differences. Values of δ_H 1.11 (3H-18) and 4.12 (H-19) for **5** and δ_H 1.27 (3H-18) and 3.92 (H-19) for **8** have been reported (both in CDCl₃)¹⁵. For the isolated compound **5** values of δ_H 1.11 (3H-18) and 4.16 (H-19) were measured. The chemical shifts of the carbon atoms CH₂-15 (δ_C 22.9), CH-19 (δ_C 72.3) and CH-21 (δ_C 59.7) reported for **5**¹³ are significantly different when compared with those reported for **8**: δ_C 28.60 (CH₂-15), 70.70 (CH-19) and 54.30 (CH-21)^{16,17}. The values measured pointed to an identical configuration for the chiral carbon C-19 of the isolated product and heyneanine (**5**) [δ_C 22.93 (CH₂-15), 71.34 (CH-19) and 59.78 (CH-21)].

By the γ-effect of the methyl group (CH₃-18) CH₂-15 (Δδ_C=5.67 ppm) in **5** and CH-21 (Δδ_C=5.40 ppm) in **8** are shielded. In the absence of this effect these carbon atoms appear deshielded [**5**: δ_C 59.87 (CH-21); **8**: δ_C 28.60 (CH₂-15)].

The new indole alkaloid **1** was isolated from the methanol extract. The ESMS showed a molecular ion at *m/z* 381 daltons ([M]⁺), in agreement with the molecular formula C₂₃H₂₉N₂O₃ and with ¹H and ¹³C NMR spectral data. This alkaloid was nearly insoluble in chloroform and its NMR spectra were therefore obtained in DMSO-*d*₆. Carbon-13 NMR experiments (HBBD and DEPT) revealed the presence of four methyl groups, four methylenes (sp³), eight methines (three sp³ and five sp²) and seven quaternary carbon atoms (one sp³ and six sp²). The ¹H-¹H-COSY, HMQC and HMBC experiments established geminal and vicinal hydrogen interactions as well as direct (¹J_{CH}) and two and three bond correlations between carbon and hydrogen atoms in the structure (Table 1). These data

Table 1. ¹H (400 MHz) and ¹³C (100 MHz) NMR for N_b-methylvoachalotine (**1**), including results obtained by heteronuclear 2D shift-correlated HMQC (¹J_{CH}) and HMBC (ⁿJ_{CH}, n=2 and 3), in DMSO-*d*₆ as solvent and residual DMSO-*d*₆ used as internal reference (δ_H 2.50 and δ_C 39.50). Chemical shifts (δ, ppm) and coupling constants (*J*, Hz, in parenthesis).*

C	¹ H- ¹³ C-HMQC- ¹ J _{CH}		¹ H- ¹³ C-HMBC- ⁿ J _{CH}	
	δ _C	δ _H	² J _{CH}	³ J _{CH}
C				
2	132.16	-	H-3	2H-6, H-14a, MeN-1
7	101.34	-	2H-6	H-5, H-9
8	124.43	-		H-6a, H-10, H-12
13	137.55	-		H-9, H-11, MeN-1
16	54.70	-	H-5, 2H-17	HO-17, 2H-6, H-14a
20	127.71	-	2H-21	3H-18
22	172.65	-		H-5, 2H-17, MeO-22
CH				
3	57.02	5.19 (br d, 11.0)		H-5, H-21b, MeN-4
5	63.16	4.19 (d, 6.4)	2H-6	2H-17, MeN-4
9	118.22	7.55 (d, 7.7)		H-11
10	119.47	7.10 (dd, 7.7, 7.1)		H-12
11	122.15	7.23 (dd, 7.1, 8.2)		H-9
12	109.82	7.51 (d, 8.2)		H-10
15	29.11	3.22		H-17a, H-19
19	118.22	5.47 (q, 6.8)	3H-18	
CH₂				
6	18.61	3.70, 3.21	H-5	
14	27.52	2.39 (ddd, 13.7, 11.0, 2.2), 2.10 (ddd, 13.7, 3.5, 3.5)		
17	62.31	3.53 (dd, 10.8, 3.6), 3.40 (dd, 10.8, 4.2)	HO-17	H-5
21	63.61	4.38 (dt, 16.4, 2.3), 4.31 (dt, 16.4, 2.0)		H-19
CH₂				
18	12.34	1.62 (d, 6.8)	H-19	
MeN-1	29.11	3.65 (s)		
MeN-4	48.70	3.16 (s)		
MeO-22	52.41	3.67 (s)		
HO-17	-	5.26 (dd, 4.2, 3.6)		

*Number of hydrogens bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-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.

revealed that **1** is closely related to voachalotine (*vide infra*), differing by the presence of an additional methyl group. The presence of the indole nucleus was clearly indicated by the ^1H and ^{13}C aromatic signals (Table 1). Typically the ^1H NMR revealed three singlet signals corresponding to methyl groups at δ_{H} 3.67, 3.65 and 3.16. Through analysis of the HMBC spectrum these signals were assigned by corresponding cross-peaks, due to heteronuclear spin-spin coupling *via* three ($^3J_{\text{CH}}$) bonds, to a carbomethoxy function and two methyl groups linked to the indole N-1 and N-4 (aliphatic N_{b}), respectively: a) C-22 (δ_{C} 172.65) with MeO-22 (δ_{H} 3.67), H-5 (δ_{H} 4.19) and 2H-17 (δ_{H} 3.53 and 3.40); b) MeN-1 (δ_{H} 3.65) with both C-2 (δ_{C} 132.16) and C-13 (δ_{C} 137.55) and c) MeN-4 (δ_{H} 3.16) with both CH-5 (δ_{C} 63.16) and CH-3 (δ_{C} 57.02). 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 voachalotine¹⁸ and the total ^1H and ^{13}C chemical shift assignments, as summarized in Table 1. The stereochemistry of the chiral carbon atom C-16 shown in **1** was clearly indicated by the chemical shift of the signal at δ_{H} 3.67 corresponding to the hydrogen of the MeO-22 (carbomethoxy group). In its epimer this signal should appear at about δ_{H} 2.50, as observed in **2** and **3** by singlet signals of the MeO-22' at δ_{H} 2.51 and 2.46, respectively⁷. Thus, the new quaternary indole alkaloid isolated from *Tabernaemontana laeta* was characterized as N_{b} -methylvoachalotine (**1**), a new natural product. Although it has been synthesized from voachalotine in a previous study¹⁸, only the ^{13}C NMR data of the iodide salt were reported.

In accordance with the revision published by Leeuwenberg⁴, the alkaloid series isolated in this study from *T. laeta* are closely related to those previously reported from *Peschiera laeta*: geissoschizol, affinine, akuammidine, normacusine B, vobasine, conodurine and voacamine^{5,6}. The similarity of the alkaloids found in this work in comparison with those from two other Brazilian *Tabernaemontana* species is remarkable. From *T. solanifolia* were reported the following alkaloids: isovoacangine, isovoacristine, coronaridine, voacangine, voacangine hydroxyindolenine, heyneanine, voacamine, voachalotine and 12-methoxy-N_b-methylvoachalotine [reported under the name *Peschiera campestris* (Rizz.) Rizz. by Gower, Pereira and Marsaioli¹⁹], while from *T. hystrix* were reported the following alkaloids: 12-methoxy-N_b-methylvoachalotine, 12-methoxy-N_b-methylvoachalotine ethyl ester and fuchsiaefoline (reported under the name *Peschiera fuchsiaefolia* by Braga and Reis¹⁸). This similarity might point to a close taxonomic relationship of these recognized species.

Experimental

General

^1H NMR and ^{13}C NMR spectra were obtained with Bruker DRX spectrometers operating at 400 and 600 MHz and 100 and 150 MHz, respectively, in CDCl_3 or $\text{DMSO}-d_6$, using the residual solvent signals as internal standard (Table 1).

Plant material

The root bark of *Tabernaemontana laeta* Mart. was collected in July 1997 at Bom Jesus do Itabapoana, 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 (1.06 kg) from *T. laeta* Mart. was extracted at room temperature using hexane and methanol, furnishing, after solvent evaporation, crude hexane (33.4 g) and methanol (120.0 g) extracts, respectively.

The hexane extract was purified by silica gel chromatography, with a gradient of EtOAc in hexane to give four fractions. The second fraction supplied large amounts (28.0 g) of a product, which by recrystallization in hexane, furnished a mixture of the four triterpenes, α -amyirin acetate, β -amyirin acetate, lupeol acetate and taraxasterol acetate. The third fraction yielded, after recrystallization, β -sitosterol (220.0 mg). The fourth fraction yielded, after column chromatography with the same system, the alkaloid voacangine¹³ (**7**, 15.0 mg).

The methanol extract was redissolved in $\text{MeOH}/\text{H}_2\text{O}$ (3:1) and extracted with CHCl_3 . The CHCl_3 layer was evaporated and further partitioned between MeOH and hexane (1:1). After another extraction with hexane, the hydromethanolic fraction was extracted with EtOAc. The methanol phase yielded 55.0 g upon evaporation, the hexane phase 22.4 g and the EtOAc phase 2.0 g.

The EtOAc extract was chromatographed on a silica gel column with a gradient of EtOAc in hexane supplying four fractions. Preparative TLC of fractions 1 and 2 (hexane:EtOAc 5%) supplied the alkaloids **5** (146.0 mg) and **6** (19.0 mg), respectively.

30.0 g of the methanol extract was chromatographed on a silica gel column and eluted with a gradient of EtOAc

in hexane, yielding four fractions; fraction 1 (1.3 g) presented additional amounts of **5**; fraction 2 (9.8 g) was rechromatographed with a gradient of CH_2Cl_2 in hexane, supplying additional amounts of **6** (0.6 g) and **7** (340.0 mg); fraction 3 (10.5 g) was rechromatographed in the same way, supplying the alkaloids **2** (130.0 mg) and **3** (315.0 mg). Fraction 4 (7.3 g) was rechromatographed with a gradient of MeOH in CH_2Cl_2 furnishing 12 fractions, of which, fractions 3-4 (520.0 mg) furnished the alkaloid **4** (53.0 mg) after rechromatography with a gradient of MeOH in CH_2Cl_2 , followed by column chromatography over Sephadex LH-20 with methanol. Fractions 6-7 (1.2 g) yielded a crystalline substance, characterized as N_b -methylvoachalotine (**1**, 38.0 mg). Fractions 10-12 (3.6 g) supplied an amorphous powder; this material (100.0 mg) was acetylated and the acetylated product was identified as 3-O- β -D-glucosyl- β -sitosterol tetraacetate.

The five alkaloids **2-6**, as well as triterpenes, β -sitosterol and 3-O- β -D-glucopyranosyl- β -sitosterol tetraacetate were identified by the analysis of the ^1H and ^{13}C NMR spectra and comparison with literature values^{7-13,15-17}.

N_b -methylvoachalotine (**1**)

White crystalline solid, mp 259-260 °C (MeOH); $[\alpha]_D^{25} - 21.3^\circ$ (DMSO, c 6.3); IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3298 (OH), 1736 (C=O), 1616, 1591, 743 (aromatic ring) (KBr); UV $\lambda_{\text{max}}/\text{nm}$ (CH_3OH) 223 (ϵ 42566), 282 (ϵ 6287); ESMS (positive ion mode) m/z 381 ($[\text{M}+\text{H}]^+$, base peak), 367 ($[\text{M}+\text{H}-\text{CH}_3]^+$); ^1H NMR and ^{13}C NMR Table 1.

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