INTRODUCTION

The genus *Rauvolfia*, family Apocynaceae, continues to be fascinating as it produces a number of indole alkaloids with novel skeletons, which are interesting from the biosynthetic point of view as well as for their medicinal aspect and spectroscopic analysis.\(^1\)\(^2\) Species of this genus have several biological activities, such as, cholinesterase inhibitors,\(^3\) and antimicrobial,\(^4\) anticonvulsant,\(^5\) antimalarial,\(^6\) antipyretic,\(^7\) and antipsychotic effects,\(^8\) in addition to sedative activity.\(^9\)

*Rauvolfia capixabae* I. Koch & Kinoshita-Gouveia,\(^10\) commonly known as “Grão-de-Gato” in Atlantic forest in the North of Espírito Santo State, appears as a tree of 6-12 m. This species has not been reported in studies on its chemical composition described in the literature.

As part of the research program for the Natural Products Chemistry Group of the North Fluminense State University (UENF) on the identification of alkaloids in species of the *Rauvolfia* genus,\(^4,11,12\) a phytochemical investigation of the stem bark extracts of *R. capixabae* is described. In the present paper, we describe the isolation and characterization of a novel sarpagine-type alkaloid, N\(_2\)-methyrauflorine (1), along with five known alkaloids. The known and new alkaloid structures were established on the basis of spectral data, mainly \(^1\)H and \(^13\)C (1D and 2D) NMR spectra, mass spectrometry and by comparison with literature data.

EXPERIMENTAL

General procedures

Optical rotation measurements were obtained on a Perkin Elmer 343 digital polarimeter. Melting points were obtained on a Microquímica MQRPF and are uncorrected. FTIR spectra were recorded on a FTIR-8300 Shimadzu spectrometer using KBr disk. EI-MS (low resolution) mass spectra were obtained on Shimadzu QP2050A mass spectrometer. Column chromatographic purifications were carried out over silica gel (70-230 mesh). Silica gel 60F\(_{254}\) was used in thin layer chromatography analysis.

\(^1\)H and \(^13\)C NMR spectra were measured on a Jeol Eclipse 400 spectrometer, operating at 400 (\(^1\)H) and 100 (\(^13\)C) MHz. CDCl\(_3\) was used as solvent and TMS as internal reference. Chemical shifts are given in the \(\delta\) scale (ppm) and coupling constants \(J\) in Hz. One dimensional (1D) \(^1\)H and \(^13\)C NMR spectra were acquired under standard conditions by using a direct detection 5 mm \(^1\)H/\(^13\)C dual probe. Standard pulse sequences were used for two dimensional spectra by using a multinuclear inverse detection 5 mm probe with field gradient.

Plant materials

The stem bark of *Rauvolfia capixabae* I. Koch & Kinoshita-Gouveia were collected in November 2004 at Cia Vale, Linhares City, Espírito Santo State, Brazil. The specimen was identified by taxonomist Ingrid Koch. A voucher specimen (CVRD 338) is deposited at the Cia Vale herbarium, Linhares, Espírito Santo State.

Extraction and isolation

The stem bark (3.08 kg) was extracted with CH\(_2\)Cl\(_2\) from *R. capixabae* I. Koch & Kinoshita-Gouveia at room temperature, furnishing 125 g of crude dichloromethane extract after solvent evaporation.

A portion of the dichloromethane extract (10.1 g) was chromatographed over silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH to afford fourteen fractions. Fraction 1 (58.4 mg) yielding isoreserpiline (2). The fraction 7 (1.35 g) was rechromatographed over a silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH yielding thirteen fractions. Fraction 7.2 (6.8 mg) was rechromatographed over a silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH yielding the compounds isoreserpiline (2), 166 mg). Fraction 7.13 (384.8 mg) was rechromatographed over a silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH yielding compound 6 (199 mg).

Fraction 8 (0.8 g) was rechromatographed over silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH supplying eight fractions. Fraction 8.1 (92.0) yielding ajmalicine (4, 92.0 mg). Fraction 8.8 (301.2) was rechromatographed over silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH yielding compound 6 (199 mg).

Fraction 11 (1.2 g) was rechromatographed over silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH supplying seven fractions. Fraction 11.7 (217.5 mg) was rechromatographed over silica gel column with a polarity gradient of CH\(_2\)Cl\(_2\)/MeOH yielding compound 6 (13.0 mg).

N\(_2\)-methyrauflorine (1), yellow amorphous solid, mp
Novel alkaloid from Rauvolfia capixabae (Apocynaceae)

3.80 was attributed to three hydrogens of a methoxyl (all linked to a nitrogen atom: CH-2, 2.64, H-5 (J = 7.0 Hz), 3H-18) was used to confirm the presence of a function carbonyl linked at atom carbon C-17, as shown in Table 1 together with additional heteronuclear long range couplings $^1$H-$^1$C-COSY-$^1$H$_{NC}$ (n=2, HMOC or HSQC and HMBC, m=3) that were also used to complete unambiguous $^1$H and $^1$C chemical shift assignments.

RESULTS AND DISCUSSION

The CHCl$_3$ extracts of R. capixabae stem bark were subjected to classical chromatographic methods to yield the a new sarpagine-type alkaloid, N$_1$-methylrauflorine (1), in addition to the known alkaloids, isoreserpiline (2), $^1$N$_2$-oxide-isoreserpiline (3), $^1$jamilicine (4), $^1$perakine (5), $^1$and vinorine (6),$^1$ that were identified in the analysis of $^1$H and APT-$^1$C NMR spectra data, including 2D $^1$H-$^1$H-COSY, $^1$H-$^1$H-NOESY, HSQC and HMBC NMR experiments,$^1$ which was confirmed by direct heteronuclear correlations ($^1$H$_{NC}$) between C-9 (δ$_h$ 121.3/δ$_n$ 6.86), C-11 (δ$_h$ 112.7/δ$_n$ 6.80), C-12 (δ$_h$ 116.0/δ$_n$ 6.84) observed in the HMBC spectrum. The presence of a single signal at δ$_n$ 3.80 was attributed to three hydrogens of a methoxyl group ($^1$H$_{NC}$ δ$_h$ 56.0/δ$_n$ 3.80), in agreement with a methoxyl group in the indole nucleus.$^1$ The single signal at δ$_h$ 3.06 (three hydrogen atoms) suggested the presence of a N-Me group that was confirmed in the HMBC by the correlation $^1$H$_{NC}$ Me-N$_1$ (δ$_h$ 37.0/δ$_n$ 3.06) and compatible with a substituted indole nucleus and N-methylated.$^1$

The new monoterpenic indole alkaloid (1) [α]$_D^{20}$ = +8.6 (CHCl$_3$, c 0.062), was obtained as a yellow amorphous solid. Comparative analysis of $^1$H- and APT-$^1$C NMR spectra (Table 1), involving the corrobororation of $^1$H NMR spectra (1D $^1$H NMR and 2D $^1$H-$^1$H-COSY), allowed to recognize the presence of 21 signals corresponding to six nonhydrogenated [(C)$_n$] one sp$^3$, five sp$^2$ (including one carbonyl group at δ$_c$ 213.6 (C-17) and one sp$^3$ olefinic at δ$_c$ 137.5 (C-20)], nine methine [(CH)$_n$] three sp$^3$ (all linked to a nitrogen atom: CH-2, CH-3 and CH-5) and four sp$^2$ including one olefinic at δ$_c$ 115.5 (CH-19), three methylene [(CH$_2$)$_n$], all sp$^3$, including one linked to a nitrogen atom at δ$_c$ 55.7 (CH2-21)] and three methyl [(CH$_3$)$_n$]; including one linked to a nitrogen atom at δ$_c$ 36.9/δ$_h$ 3.06, one linked to an sp$^2$ olefinic carbon atom at δ$_c$ 12.9/δ$_h$ 1.65 and methoxyl group (δ$_c$ 56.0/δ$_h$ 3.80),$^1$ carbon atoms, allowing to deduce the expanded molecular formula (C$_2$H$_2$N$_2$O$_2$)$_n$ for 1.

The LREI-MS (70 eV) spectrum of 1 (Scheme 1) showed of molecular peak [M$^+$] at m/z 336 Daltons, allowing in conjugation with the $^1$C NMR spectral data to deduce the molecular formula C$_{21}$H$_{20}$N$_{14}$O$_2$ (1), containing eleven degrees of unsaturation and consistent with the presence of one carbonyl group and one double bond in a methoxylated pentacyclic alkaloid, compatible with the alkaloidic structure sustaining a carbonyl group at carbon atom C-17 (δ$_c$ 213.6) in rauflorine skeleton (1a).$^1$ In fact, heteronuclear long-range couplings ($^1$J$_{NC}$) of this carbon atom C-17 (δ$_c$ 213.6) with H-2 (δ$_h$ 2.64), H-5 (δ$_h$ 3.18-3.24) and H-6a (δ$_h$ 2.38) was used to confirm the presence of a function carbonyl linked at atom carbon C-17, as shown in Table 1 together with additional heteronuclear long range couplings $^1$H-$^1$C-COSY-$^1$H$_{NC}$ (n=2, HMQC or HSQC and HMBC, m=3) that were also used to complete unambiguous $^1$H and $^1$C chemical shift assignments.

Figure 1. Compounds Isolated from R. Capixabae

166-168 °C; [α]$_D^{20}$ = + 8.06° (CHCl$_3$, c 0.062); LREI-MS (rel. int.) 336 [M$^+$ (100%,)] (Scheme 1); $^1$H and $^1$C NMR: see Table 1.
All these data allowed the definition of a sapargine-type skeleton and propose the Structure for the alkaloid isolated. The comparison of the $^1$H and $^{13}$C NMR spectral data of 1 with values described in the literature for the rauflorine alkaloid (1a) allowed to verify that the significant difference between these two alkaloids can be justified by presence of the methyl group linked to N-atom of the alkaloid 1.

The relative stereochemistry proposed for alkaloid 1 was based on biogenic argument, by comparison with data described in the literature for sapargine-type alkaloids skeleton and results revealed by dipolar-dipolar interactions observed in a $^1$H-$^1$H-NOESY experiment (Table 1).

**CONCLUSION**

From dichloromethane extract of *R. capixaba* stem bark a new sarpagine-type alkaloid was isolated, $N_a$-methylrauflorine (1), in addition to known alkaloids, isoreserpiline (2), $N_b$-oxide-isoreserpiline (3), ajmalicine (4), perakine (5) and vinorine (6).

**SUPPLEMENTARY MATERIAL**

The LREIMS, $^1$H NMR, $^{13}$C NMR –APT, $^1$H-$^1$H COSY NMR, HMOC, HMBC and NOESY spectra for compound 1 are freely available at http://quimicanova.sbq.org.br in PDF file.
Table 1. $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR data, including results obtained by heteronuclear long-range couplings observed in HMQC and HMBC spectra, in CDCl$_3$ as solvent. The chemical shifts in $\delta$ (ppm) and coupling constants ($J$, in parenthesis) in Hz.

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<th>C</th>
<th>$\delta_1$</th>
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<th>$^2J_{HC}$</th>
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<tr>
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*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 from 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-NOESY spectra.

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REFERENCES