http://dx.doi.org/10.5935/0100-4042.20160002 *Quim. Nova,* Vol. 39, No. 2, 156-159, 2016

Artigo

NOVEL ALKALOID FROM *Rauvolfia capixabae* (APOCYNACEAE)

Lanamar Almeida Carlos[a,*], Leda Mathias[b], Raimundo Braz-Filho[b] and Ivo Jose Curcino Vieira[b]
[a]Departamento de Engenharia de Alimentos, Universidade Federal de São João del-Rei, Campus Sete Lagoas, 35701-970 Sete Lagoas – MG, Brasil
[b]Laboratório de Ciências Químicas, Centro de Ciencias e Tecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 28013-602 Campos dos Goytacazes – RJ, Brasil

Recebido em 15/07/2015; aceito em 29/09/2015; publicado na web em 14/01/2016

A new sarpagine-type alkaloid, N_a-methylrauflorine (**1**), was isolated from *Rauvolfia capixabae* together with isoreserpiline (**2**), N_b-oxide-isoreserpiline (**3**), ajmalicine (**4**), perakine (**5**) and vinorine (**6**) alkaloids. These compounds were characterized based on their spectral data basis, mainly one- (^{1}H, ^{13}C, APT) and two-dimensional (^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, HMQC and HMBC) NMR, and mass spectra, also involving comparison with data from the literature.

Keywords: Apocynaceae; *Rauvolfia capixabae*; indole alkaloids.

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] anxiolytic,[6] antimalarial, 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_a-methylrauflorine (**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 QP5050A 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 δ 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_2Cl_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_2Cl_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_2Cl_2/MeOH yielding thirteen fractions. Fraction 7.2 (6.8 mg) presented as an amorphous yellow solid identified as Compound (**1**). Fraction 7.13 (384.8 mg) was rechromatographed over a silica gel column with a polarity gradient of CH_2Cl_2/MeOH yielding the compounds isoreserpiline (**2**, 7.1 mg) and perakine (**5**, 166 mg).

Fraction 8 (0.8 g) was rechromatographed over silica gel column with a polarity gradient of CH_2Cl_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_2Cl_2/MeOH yielding compound **6** (199 mg).

Fraction 11 (1.2 g) was rechromatographed over silica gel column with a polarity gradient of CH_2Cl_2/MeOH supplying seven fractions. Fraction 11.7 (217.5 mg) was rechromatographed over silica gel column with a polarity gradient of CH_2Cl_2/MeOH yielding compound N_b-oxide isoreserpiline (**3**, 13.0 mg).

N_a-methylrauflorine **(1)**, yellow amorphous solid, mp

*e-mail: lanamar@ufsj.edu.br

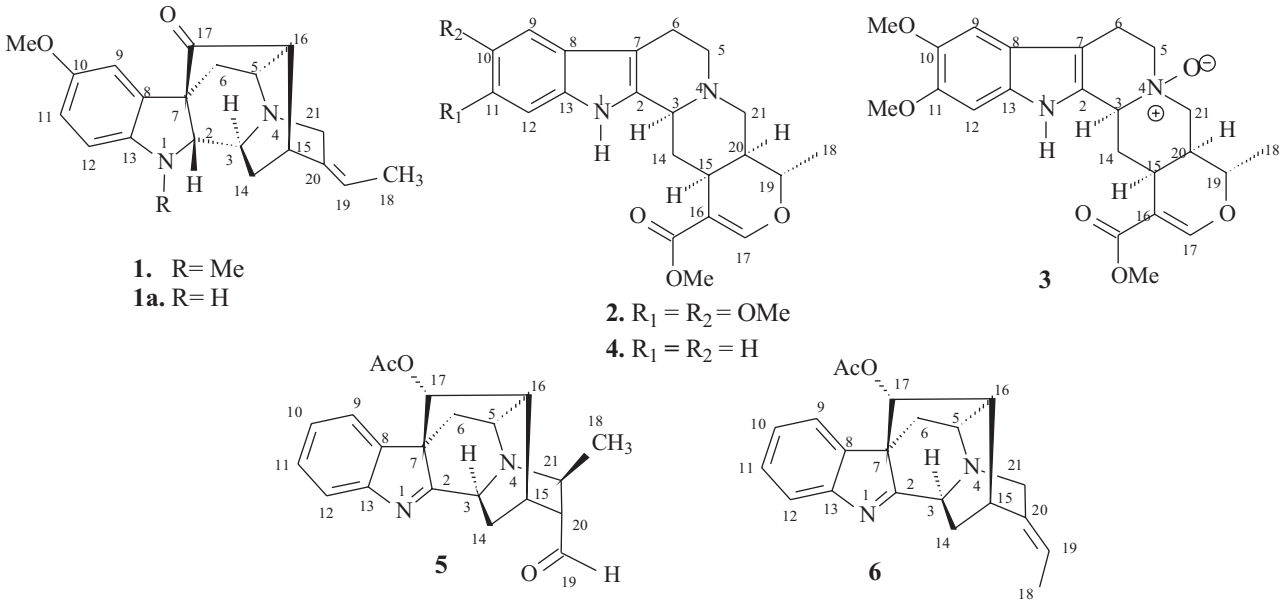


Figure 1. *Compounds Isolated from R. Capixabae*

166-168 °C; $[\alpha]_D^{23}$ = + 8.06° ($CHCl_3$, *c* 0.062); LREI-MS (rel. int.) 336 [$M^{+\cdot}$ (100%,)] (Scheme 1); ^1H and ^{13}C NMR: see Table 1.

RESULTS AND DISCUSSION

The CH_2Cl_2 extracts of *R. capixabae* stem bark were subjected to classical chromatographic methods to yield the a new sarpagine-type alkaloid, N_a-methylrauflorine (**1**), in addition to the known alkaloids, isoreserpiline (**2**),[11] N_b-oxide-isoreserpiline (**3**),[12] ajmalicine (**4**),[13] perakine (**5**),[14] and vinorine (**6**),[15] that were identified in the analysis of ^1H and APT-^{13}C NMR spectra data, including 2D ^1H-^1H-COSY, ^1H-^1H-NOESY, HSQC and HMBC NMR experiments,[16,17] which were also used to complete unambiguous ^1H and ^{13}C chemical shift assignments of the new alkaloid **1**.

The new monoterpenic indole alkaloid (**1**) [a]$_D^{23}$ = + 8.6, ($CHCl_3$, *c* 0.062), was obtained as a yellow amorphous solid. Comparative analysis of {^1H}- and APT-^{13}C NMR spectra (Table 1), involving the corroboration of ^1H NMR spectra (1D ^1H NMR and 2D ^1H-^1H-COSY), allowed to recognize the presence of 21 signals corresponding to six nonhydrogenated [$(C)_6$: one sp^3, five sp^2 (including one carbonyl group at δ_C 213.6 (C-17) and one sp^2 olefinic at δ_C 137.5 (C-20)], nine methine [$(CH)_9$: 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 [$(CH_2)_3$, all sp^3, including one linked to a nitrogen atom at δ_C 55.7 (CH2-21)] and three methyl [$(CH_3)_3$: 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,*s*)] carbon atoms, allowing to deduce the expanded molecular formula $(C)_5(C{=}O)(CH)_9(CH_2)_3N(CH_3)(NCH_3)(OCH_3)$ = $C_{21}H_{24}N_2O_2$ for **1**.

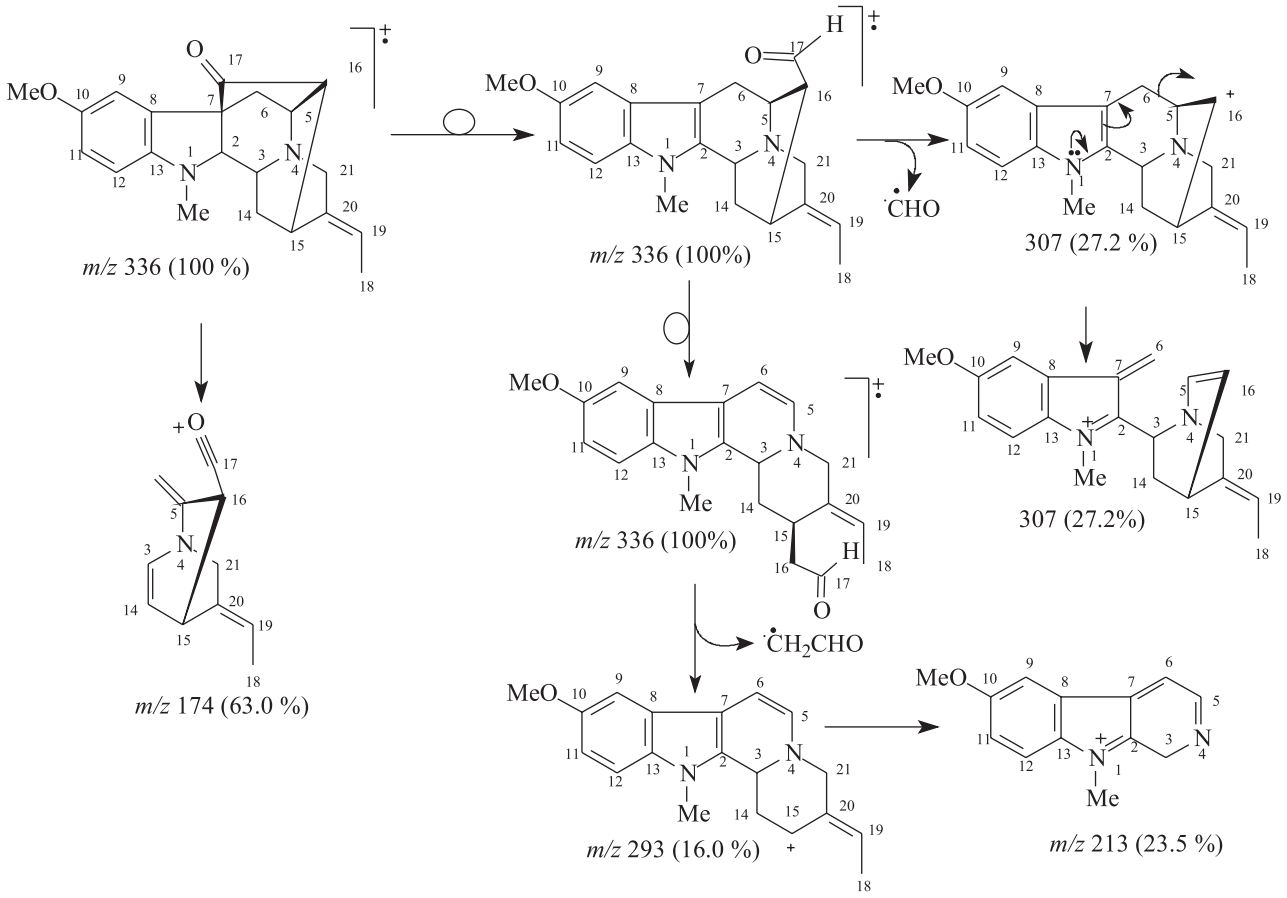
The LREI-MS (70 eV) spectrum of **1** (Scheme 1) showed of molecular peak [$M^{+\cdot}$] at *m/z* 336 Daltons, allowing in conjugation with the ^{13}C NMR spectral data to deduce the molecular formula $C_{21}H_{24}N_2O_2$ (**1**), containing eleven degrees of unsaturation and consistent with the presence 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**).[4] In fact, heteronuclear long-range couplings ($^3J_{HC}$) 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 ^1H-^{13}C-COSY-$^nJ_{HC}$ (n=2, HMQC or HSQC and HMBC, n=3) that were also used to complete unambiguous ^1H and ^{13}C chemical shift assignments.

^1H NMR spectrum of **1** showed three signals corresponding to the hydrogens linked to aromatic carbons at δ_H 6.86 (*br s*, H-9), 6.84 (*d, J* = 9.2 Hz, H-12) and 6.80 (*dd*, *J* = 9.2 and 3.0 Hz, H-1) typical of a 1,2,4-trisubstituted benzene ring (ring A),[18,19] which was confirmed by direct heteronuclear correlations ($^1J_{HC}$) between CH-9 (δ_C 121.3)/δ_H 6.86), CH-11 (δ_C 112.7/δ_H 6.80), C-12 (δ_C 116.0/δ_H 6.84) observed in the HMQC spectrum. The presence of a single signal at δ_H 3.80 was attributed to three hydrogens of a methoxyl group ($^1J_{HC}$ δ_C 56.0/δ_H 3.80), in agreement with a methoxyl group in the indole nucleus.[18,19] The single signal at δ_H 3.06 (three hydrogen atoms) suggested the presence of a *N*-Me group that was confirmed in the HMQC by the correlation $^1J_{HC}$ Me-N_a (δ_C 37.0/ δ_H 3.06) and compatible with a substituted indole nucleus and *N*-methylated.[20]

The location of the methoxyl and Me-N_a groups in the indole moiety was confirmed by heteronuclear long-range couplings observed in the HMBC spectrum by correlations $^3J_{HC}$ between by C-8 (δ_C 131.1) and H-12 (δ_H 6.84), C-10 (δ_C 147.5), H-12 (δ_H 6.84) and MeO-10 (δ_H 3.80), C-13 (δ_C 141.9), H-9 (δ_H 6.86), H-11 (δ_H 6.80) and Me-N_a (δ_H 3.06), CH-11 (δ_C 112.7) and H-9 (δ_H 6.86), and CH-2 (δ_C 79.5) and Me-N_a (δ_H 3.06), shown in Table 1.

The presence of a methyl group bonded to a sp^2 methine carbon was recognized by a double signal at δ_H 1.65 (*d*, *J* = 7.0 Hz, 3H-18) and the multiplet at δ_H 5.29-5.32 (*m*, H-19), characterizing a C=CH-CH_3 moiety corresponding to CH-19 and CH_3-18, respectively, present in several alkaloids. This moiety was further confirmed by long-range correlations revealed by the HMBC spectrum through correlations observed between the CH_3-18 (δ_C 12.9) and H-19 (δ_H 5.29-5.32, $^2J_{HC}$), CH-19 (δ_C 115.5), 3H-18 (δ_H 1.65, $^2J_{HC}$) and C-20 (δ_C 137.5) and 3H-18 (δ_H 1.65, $^3J_{HC}$).[18,19,21]

The presence of a carbonyl carbon at C-17 was confirmed by the heteronuclear correlations observed in the HMBC spectrum, which revealed long-range correlations between C-17 (δ_C 213.6) and H-16 (δ_H 2.58, $^3J_{HC}$), H-2 (δ_H 2.64, $^3J_{HC}$), H-5 (δ_H 3.22, $^3J_{HC}$) and H-6a (δ_H 1.66, $^3J_{HC}$).[18,19,21] The other correlations are shown in Table 1.



Scheme 1. *Proposed fragmentation mechanisms to justify principal peaks observed in the mass spectrum (LREIMS, 70 eV) of* ***1***

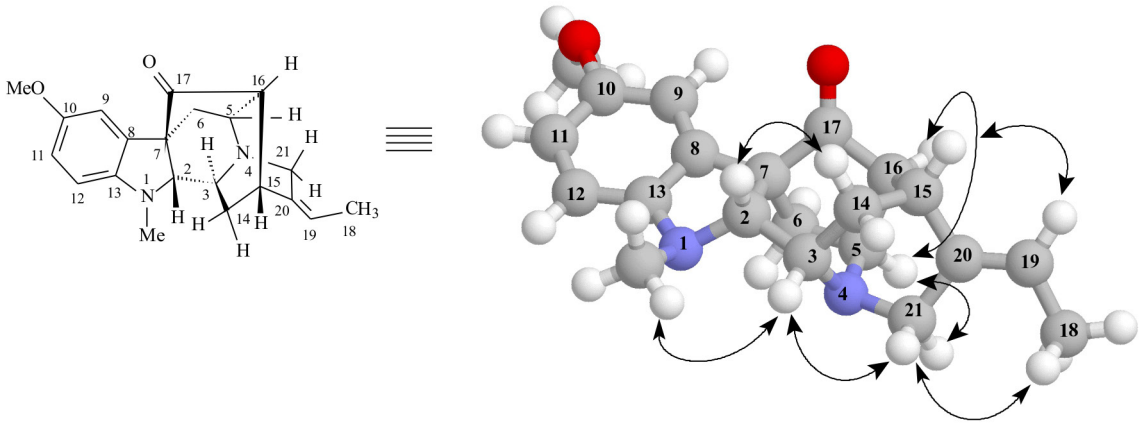


Figure 2. *Select dipolar-dipolar interactions revealing spatial correlations (NOE) and relative stereochemistry for Alkaloid 1. Arrows denote NOE spatial correlations principals*

All these data allowed the definition of a sapargine-type skeleton[18,19,21] and propose the Structure **1** 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**)[18] 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,[22] by comparison with data described in the literature for sapargine-type alkaloids skeleton[18-21] and results revealed by dipolar-dipolar interactions observed in a ^{1}H-^{1}H-NOESY experiment (Table **1**).

CONCLUSION

From dichloromethane extract of *R. capixabae* 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, HMQC, 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 δ (ppm) and coupling constants (*J,* in parenthesis) in Hz

C	HMQC		HMBC		^{1}H-^{1}H-NOESY
	δ_C	δ_H	$^{2}J_{HC}$	$^{3}J_{HC}$	
2	79.5	2.64 (*br s*)	H-3	Me-N_a; 2H-6; 2H-14	Me-*N*-1 H-14β
3	49.5	3.68 (*d*, 9.9)		H-15; 2H-21	
5	53.1	3.18 (*m*)	H-6a	H-3; H-15; 2H-21	H-21
6	35.3	2.38 (*d*, 12.1) 1.66 (*m*)		H-2	
7	58.0	-	H-2; 2H-6	H-3; H-5	
8	131.0	-		H-12	
9	121.3	6.86 (*s*)			
10	147.5	-		H-12; MeO-10	
11	112.7	6.80 (*dd*, 9.2; 3.0)		H-9	
12	116.0	6.84 (*d*, 9.2)			
13	141.9	-		H-9; H-11; Me-N_a	
14	31.6	1.91 (*m*) 1.41 (*dd*, 13.9; 4.4)	H-3	H-2; H-16	H-21α; H-2β
15	28.6	3.17 (*m*)	2H-14; H-16	H-3; H-19	
16	50.3	2.58 (*br t*, 5.1)		H-6a; H14a	H-5
17	213.6	-	H-16	H-2; H-5; H-6a	
18	12.9	1.65 (*d*, 7.0)	H-19		2H-21
19	115.5	5.30 (*m*)	3H-18	H-15; 2H-21	H-15
20	137.5	-	H-15; 2H-21	2H-14; 3H-18	
21	55.7	3.50 (*br s*)		H-5; H-19	H-3; H-5; H-14α; 3H-18
MeO-10	56.4	3.80 (*s*)			
Me*N*-1	36.9	3.06 (*s*)		H-2	H-2; H-3

*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-COSY spectra.

ACKNOWLEDGEMENTS

The authors are grateful to Fundação Carlos Chagas Filho de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), Consenho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for scholarships and financial support.

REFERENCES

1. Roberts, M. F.; Wink, M.; *Alkaloids: Biochemistry, Ecology and Medicinal Applications*; Plenum Press: New York, 1998.
2. Bruneton, J.; *Pharmacognosy, Phytochemistry, Medicinal Plants*, Lavoisier: Londres, 1995.
3. Tappayuthpijarn, P.; Jansom, C.; Jansom, V.; Ingkaninun, K.; *Planta Med.* **2010**, *76*, 1209.
4. Carlos, L. A.; Silva, A. K. A.; Vieira, I. J. C.; Mathias, L.; Braz-Filho, R.; Samarão, S. S.; Vieira-da-Motta, O.; *Braz. J. Microbiol*. 2010, *41*, 612.
5. Quintans-Junior, L. J.; Siqueira, J. S.; Melo, M. S.; Silva D. A.; Morais, L. C. S. L.; Souza, M. F. V.; Almeida, R. N.; *Rev. Bras. Farmacogn.* **2010**, *20*, 54.
6. Netto, S. M.; Warela R. W. B.; Fechine, M. F.; Queiroga, M. N.; Quintans-Júnior, L.; *J. Rev. Bras. Farmacogn.* **2009**, *19*, 888.
7. Amole, O. O.; Onabanjo, A. O.; Agbaje, E. O.; *Arch. Pharmacol*. **1998**, *358*, 497.
8. Obembe, A.; Sokomba, E. N.; Sijuwola, O. A.; Olorunfemi, P. O.; Alemika, T. O. E.; Isichei, C. O.; Ogunkeye, O. O.; Bangudu, A. B.; *Phytother. Res*. **1994**, *8*, 218.
9. Madawala, P. G.; Arambewela, L. S. R.; Premakumara, G. A. S.; Ratnasooria, W. D.; *J. Ethnopharmacol.* **1994**, *42*, 63.
10. Koch, I.; Kinoshita, L. S.; Bittrich, V.; *Novon* **2007**, *17*, 462.
11. Cancelieri, N. M.; Vieira, I. J. C.; Mathias, L.; Schripsema, J.; Braz-Filho, R.; *Tetrahedron Lett.* **2002**, *43*, 1783.
12. Cancelieri, N. M.; Vieira, I. J. C.; Mathias, L.; Braz-Filho, R.; *Magn. Reson. Chem*. **2003**, *41*, 287.
13. Lounasmaa, M.; Tolvanem, A.; *Heterocycles* **1986**, *24*, 3229.
14. Rahman, A.-U.; Sultana, A.; Nighat, F.; Bhatti, K.; Kuruku, S.; Kartal, M.; *Phytochemistry* **1995**, *38*, 1057.
15. Kato, L.; Braga, R. M.; Kock, I.; Kinoshita, L. S.; *Phytochemistry*, **2002**, *60*, 315.
16. Claridge, T. D. W.; *High-Resolution NMR Techniques in Organic Chemistry*, Pergamon: Amsterdam, 1999.
17. Friebolin, H.; *Basic One and Two-Dimensional NMR Spectroscopy*, 2nd ed., VCH: New York, 1993.
18. Jokela, R.; Lounasmaa, M. A.; *Heterocycles* **1996**, *43*, 1015.
19. Chatterjee, A.; Ghosh, A. K.; Hagaman, E. W.; *J. Org. Chem.* **1982**, *47*, 1732.
20. Azoug, M.; Loucaci, A.; Richard, B.; Nuzillard, J.; Moreti, C.; Hanrot, Z.; Men-Oliver, L.; *Phytochemistry* **1995**, *39*, 1223.
21. Braga, R. M.; Reis, F. A. M.; *Phytochemistry* **1987**, *26*, 833.
22. Danieli, B.; Palmisano, G.; In *The Alkaloids*; Brossi, A., ed.; Academic Press: New York, 1986.