J. Braz. Chem. Soc., Vol. 16, No. 3B, 553-557, 2005.
Printed in Brazil - ©2005 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Muscicapines, a New Class of Guaiane-Type Sesquiterpene Alkaloids from *Croton muscicapa*

Vicente T. de Araújo-Júnior[a], Marcelo S. da Silva[a], Emídio V. Leitão da-Cunha[a,b], Maria de Fátima Agra[a], Petrônio F. de Athayde-Filho[a], Ivo J. Curcino Vieira[c], Raimundo Braz-Filho[c], and José M. Barbosa-Filho[*,a]

[a]*Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, CP 5009, 58051-970 João Pessoa - PB, Brazil*

[b]*Departamento de Farmácia e Biologia, Universidade Estadual da Paraíba, 58100-000 Campina Grande - PB, Brazil*

[c]*Setor de Química de Produtos Naturais-LCQUI, Universidade Estadual do Norte Fluminense, 28015-620 Campos - RJ, Brazil*

Três novos alcalóides sesquiterpênicos do tipo guaiano, muscicapina A (**1**), muscicapina B (**2**) e muscicapina C (**3**) foram isolados das raízes de *Croton muscicapa*. As estruturas foram elucidadas através de análises espectroscópicas, principalmente RMN de 1D e 2D e espectrometria de massas. Este é o primeiro relato na literatura de uma nova classe de alcalóides sesquiterpênicos com esqueleto guaiano.

Three new guaiane-type sesquiterpene alkaloids, muscicapine A (**1**), muscicapine B (**2**), and muscicapine C (**3**) were isolated from the roots of *Croton muscicapa*. The structures were established by analysis of spectroscopic data, mainly 1D and 2D NMR and MS. This is the first report of a new class of guaiane-type sesquiterpene alkaloids.

Keywords: *Croton muscicapa*, Euphorbiaceae, guaiane sesquiterpene alkaloid, muscicapines

Introduction

Plants of the genus *Croton* (Euphorbiaceae), widely distributed throughout tropical areas, are used in South America as sources of traditional medicines for the treatment of wounds, inflammation, and cancer.[1] Their species are known to be rich in terpenoids (essential oils and diterpenes) and alkaloids (indole and mainly isoquinoline derivatives).[2]

In a search for new drugs from plants of the genus *Croton*, a previous work described the isolation of clerodane and labdane diterpenes from *Croton polyandrus* Spreng[3] and diterpenes and alkaloids from *Croton moritibensis* Baill.[4] In this work, the chemical investigation of *Croton muscicapa* Müll. Arg., a native shrub to Northeastern Brazil, popularly known as "velame de cheiro", is reported. From the ethanolic extract of the roots, four alkaloids (**1-4**) were isolated through a series of partitions followed by chromatographic procedures.[5] The alkaloids isolated were the known nicotine derivative anabasine (**4**) and three new guaiane-type sesquiterpene alkaloids **1**, **2** and **3** which are described here for the first time (Figure 1).

The literature on components of essential oil refers to an enormous number of lower terpenes, but no mono- and

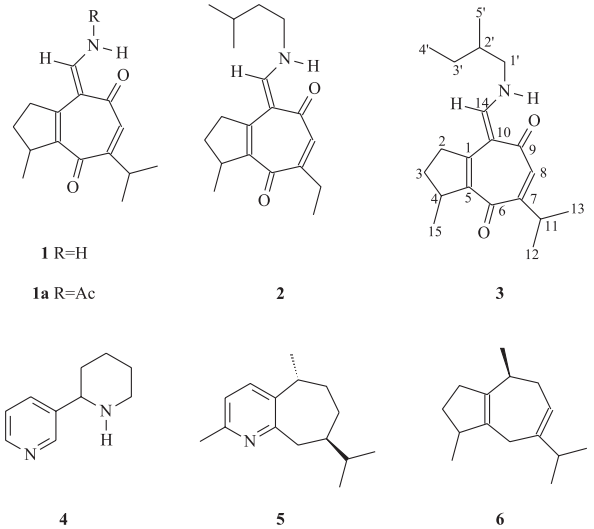


Figure 1.

* e-mail: jbarbosa@ltf.ufpb.br

sesquiterpene alkaloids. However, alkaloids derived from diterpenes and steroids are widespread in plants. To the best of our knowledge the alkaloid with the carbon skeleton closest to the guaiane-type sesquiterpene alkaloids is epiguaipyridine (**5**) isolated from the essential oil of *Pogostemon pachouli*.[6]

Results and Discussion

The known alkaloid anabasine (**4**) was identified by spectral data, mainly ^{1}H and ^{13}C NMR spectra, and comparison with literature values.[7]

Alkaloid **1** was obtained as an amorphous yellow solid and showed $[\alpha]_D^{20}$ – 44° ($CHCl_3$, *c* 0.05). The IR spectrum revealed bands at ν_{max} 3366 (N-H), 1650 (conjugated carbonyl group stretching) and 2932-2833 cm^{-1} (C-H stretching).[8] The EIMS of **1** (Scheme 1) showed a molecular ion peak at *m/z* 245 daltons ($[M]^{\bullet+}$) which, together with ^{1}H and ^{13}C (HBBD and APT) NMR spectral data (Tables 1 and 2), were in agreement with the molecular formula $C_{15}H_{19}NO_2$, compatible with a sesquiterpenoid skeleton with an additional nitrogen atom.[6]

The ^{1}H and ^{13}C NMR spectral data of **1**, including ^{1}H-^{13}C-COSY-$^nJ_{CH}$ (n=1, HMQC; n=2 and 3, HMBC) (Table 2), are in agreement with a guaiane sesquiterpene skeleton oxidized with the formation of an enamine group between C-10 (δ_C 110.30) and C-14 (δ_C 159.27). The location of the enamine group at C-10 (δ_C 110.30) was unequivocally deduced by HMBC correlations between H-14 (δ_H 7.89) and carbons C-10 (δ_C 110.30, $^2J_{CH}$) and C-9 (δ_C 188.32, $^3J_{CH}$). The presence of a primary enamine was suggested by the correlations of H-14 (δ_H 7.89, dd, *J* 8.2 and 13.9 Hz) and amino group NH_2 (δ_H 11.71) in the ^{1}H-^{1}H-COSY spectrum. The 2-ene-1,4-dione system was confirmed by HMBC correlations between the carbonyl carbon C-9 (δ_C 188.32,) and hydrogens H-14 (δ_H 7.89, $^3J_{CH}$) and H-8 (δ_H 6.76, $^2J_{CH}$) (Table 2). The isopropyl group, was characterized by ^{1}H NMR spectra (1D and 2D ^{1}H-^{1}H-COSY) by two doublets (*J* 6.7 Hz) at δ_H 1.15 and 1.14 (H_3-12 and H_3-13) and a multiplet at δ_H 3.38 (H-11). This together with HMQC (CH_3-12 and CH_3-13: δ_H/δ_C 1.15/22.84 and 1.14/22.41; CH-11: δ_H/δ_C 3.38/30.42) and HMBC correlations (Table 2) of C-7 with both methyl groups H_3-12 (δ_H 1.15, $^3J_{CH}$) and H_3-13 (δ_H 1.14, $^3J_{CH}$), H-8 (δ_H 6.76, $^2J_{CH}$) and H-11 (δ_H 3.38, $^2J_{CH}$) confirm the presence of the isopropyl group at C-7. The presence of the methyl group CH_3-15 at position C-4, was determined by the doublet signal at δ_H 1.17 (*J* 6.9 Hz) observed in the ^{1}H (1D and 2D ^{1}H-^{1}H-COSY) NMR spectra (Table 1) and by the HMBC correlations with the quaternary sp^2 carbon C-5 (δ_C 139.73) (Table 2). Additional heteronuclear long-range correlations observed in the HMBC are summarized in Table 2.

The alkaloids **2**, $[\alpha]_D^{20}$ – 6° ($CHCl_3$, *c* 0.02), and **3**, $[\alpha]_D^{20}$ – 9° ($CHCl_3$, *c* 0.05), were separated by recycling HPLC using reverse phase silica gel. Analysis of the spectral data of **1**, **2,** and **3** allowed their identification as guaiane sesquiterpene alkaloids containing the common basic skeleton. The EIMS of isomers **2** and **3** (Scheme 1) showed molecular ion peaks at *m/z* 315 daltons ($[M]^{\bullet+}$), which, together with ^{1}H and ^{13}C (HBBD and APT) NMR spectral data (Table 2), suggested the deduction of the molecular formula $C_{20}H_{29}NO_2$. Comparison of this molecular formula with that of **1** ($C_{15}H_{19}NO_2$) suggested the presence of an additional reduced isoprene moiety C_5H_{10} ($C_{20}H_{29}NO_2$ - $C_{15}H_{19}NO_2$ = 70 daltons) in **2** and **3**.

The isopentyl group bonded to the nitrogen atom of **2** was characterized as 3-methylbutyl by the doublet signal at δ_H 0.96 (*J* 6.6 Hz, H_3-4' and H_3-5') and multiplet signals at δ_H 1.74 (H-3'), 1.60 (H_2-2') and 3.50 (H_2-1') in the ^{1}H (1D and 2D ^{1}H-^{1}H-COSY) NMR, which showed heteronuclear correlations with ^{13}C signals at δ_C 22.28 (CH_3-4' and CH_3-5'), 25.64 (CH-3'), 39.47 (CH_2-2'), and 49.13 (CH_2-1') in the HMQC spectrum (Table 1). The location of this group at the nitrogen atom [*N*-(3-methylbutyl) derivative of **1**] was suggested by the ^{1}H and ^{13}C chemical shifts of the methylene group CH_2-1' (δ_H 3.50 and δ_C 49.13) and confirmed by HMBC correlations between CH-14 (δ_C 160.40) and 2H-1' (δ_H 3.50, $^3J_{CH}$) (Table 2). Additional HMBC correlations are indicated in Table 2.

The presence of a 2-methylbutyl group in **3** was deduced by the signals at δ_H 1.01 (d, *J* 6.9 Hz, H_3-5'), 0.95 (t, *J* 7.7, H_3-4'), 1.27 (m, H_2-3'), 1.48 (m, H-2'), and 3.38/3.31 (m, H_2-1') in the ^{1}H (1D and 2D ^{1}H-^{1}H-COSY) NMR spectra, which was confirmed by the HMQC and HMBC spectra (Tables 1 and 2). HMBC correlations between CH-14 (δ_C 160.87) and H_2-1' (δ_H 3.38 and 3.31), together with the ^{13}C chemical shift of the methylene group CH_2-1' (δ_C 56.91) (Table 2) were used to locate the 2-methylbutyl group at the nitrogen atom [*N*-(2-methylbutyl) derivative of **1**]. Additional heteronuclear long-range couplings are summarized in Table 2. The alkaloids were named muscicapine A, muscicapine B, and muscicapine C, respectively.

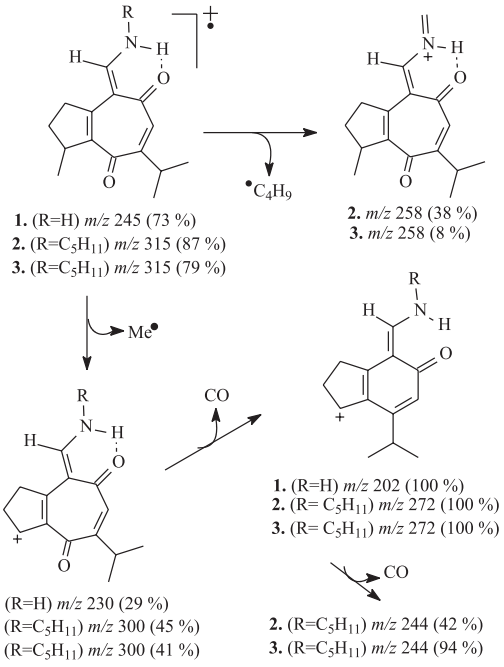
To our knowledge, the muscicapines represent the first members of a new class of guaiane-type sesquiterpene alkaloids in plants. Biogenetically, their precursor should be the guaiadiene **6**, which had the CH_3-14 oxidized to the corresponding aldehyde, which, after reductive amination, incorporated the amino groups. The introduction of a double bond between C-10 and C-14 by NADP/FAD and oxidation of C-6 and C-9 furnishes muscicapine A (**1**). The other two muscicapines B (**2**) and C (**3**) probably originate by condensation of the aldehyde with the corresponding

3-methyl and 2-methyl butylamines probably originated by descarboxylation of leucine and isoleucine, respectively.

Experimental

General procedures

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker NMR spectrometer (DRX 500) with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.27 and 77.0 ppm) were used as references. The chemical shifts (δ) are given in ppm, and the coupling constant (J) in Hz. All programs used for performing the 2D NMR experiments are part of the Bruker library. EIMS data were determined on a JEOL mass spectrometer using direct insertion probe at 70 eV. IR spectra were obtained using KBr pellets in a Shimadzu Infrared Spectrometer model FTIR-8300. The optical rotation [α] value was obtained



Scheme 1. Proposed fragmentation mechanisms of **1**, **2** and **3** (only principals peaks).

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR for muscicapine A (**1**), *N*-acetylmuscicapine (**1a**), muscicapine B (**2**), and muscicapine C (**3**), in $CDCl_3$ as solvent

	1		**1a**		**2**		**3**	
C	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	146.99	-	144.48	-	147.03	-	147.03	-
5	139.73	-	143.65	-	139.65	-	139.65	-
6	186.56	-	183.67	-	185.55	-	185.55	-
7	157.14	-	165.29	-	157.86	-	157.86	-
9	188.32	-		-	186.54	-	186.54	-
10	110.30	-	121.75	-	109.16	-	109.16	-
Ac	-	-	172.88	-	-	-	-	-
CH								
4	42.53	3.42 (m)	42.00	3.58-3.30 (m)	42.14	3.44 (m)	42.14	3.44 (m)
8	135.42	6.76 (s)	132.71	6.57 (s)	135.37	6.83 (s)	135.37	6.82 (s)
11	30.42	3.38 (m)	31.16	3.58-3.30 (m)	30.32	3.40 (m)	30.32	3.40 (m)
14	159.27	7.89 (dd, 8.2, 13.9)	146.34	8.07 (d, 11.4)	160.40	7.73 (d, 13.2)	160.87	7.70 (d, 13.2)
2'	-	-	-	-	-	-	35.95	1.48 (m)
3'	-	-	-	-	25.64	1.74 (m)	-	-
CH_2								
2	35.88	2.96 (td, 15.9, 8.1) 2.79 (ddd, 15.9, 9.1, 4.3)	35.00	3.26 (m)3.12 (m)	36.01	3.00 (m)2.87 (m)	36.01	3.00 (m)2.87 (m)
3	30.10	2.06 (m); 1.53 (m)	30.24	2.85 (m); 1.64 (m)	30.19	2.07 (m); 1.55 (m)	30.19	2.07 (m); 1.55 (m)
1'	-	-	-	-	49.13	3.50 (m)	56.91	3.38 (m); 3.31 (m)
2'	-	-	-	-	39.47	1.60 (m)	-	-
3'	-	-	-	-	-	-	26.80	1.27 (m)
CH_3								
12	22.84	1.15 (d, 6.7)	22.52	1.20 (d, 6.6)	22.65	1.13 (d, 6.6)	22.65	1.13 (d, 6.6)
13	22.41	1.14 (d, 6.7)	22.07	1.18 (d, 6.6)	22.23	1.13 (d, 6.6)	22.23	1.13 (d, 6.6)
15	19.53	1.17 (d, 6.9)	18.63	1.20 (d, 6.2)	19.23	1.16 (d, 7.0)	19.23	1.16 (d, 7.0)
4'	-	-	-	-	22.28	0.96 (d, 6.6)	11.38	0.95 (t, 7.7)
5'	-	-	-	-	22.28	0.96 (d, 6.6)	17.13	1.01 (d, 6.9)
H_2N	-	11.71 (s)	-	-	-	-	-	-
HN		-	-	12.67 (brd)	-	12.93 (brs)	-	13.01 (brs)
Ac	-	-	21.74	1.89 (s)	-	-	-	-

Table 2. Long-range correlations observed in the HMBC ($^nJ_{CH}$, n=2 and 3) spectra of muscicapine A (**1**), muscicapine B (**2**), and muscicapine C (**3**), in $CDCl_3$ as solvent and residual $CDCl_3$[a]

	1			**2**			**3**		
C	δ_C	$^2J_{CH}$	$^3J_{CH}$	δ_C	$^2J_{CH}$	$^3J_{CH}$	δ_C	$^2J_{CH}$	$^3J_{CH}$
1	146.99	2H-2	H-14	147.03	2H-2	H-14	147.03	2H-2	H-14
5	139.73		2H-3; 3H-15	139.65		2H-3; 3H-15	139.65		2H-2; 3H-15
6	186.56			185.55		H-8	185.55		H-8
7	157.14	H-8; H-11	3H-12; 3H-13	157.86	H-8; H-11	3H-12; 3H-13	157.86	H-8; H-11	3H-12; 3H-15
9	188.32	H-8	H-14	186.54		H-14	186.54		H-14
10	110.30	H-14	H-8	109.16	H-14	H-8	109.16	H-14	H-8
CH									
4	42.53	2H-3; 3H-15	2H-2	42.14	3H-15		42.14	3H-15	
8	135.42		H-11	135.37		H-11	135.37		H-11
11	30.42	3H-12; 3H-13		30.32	3H-12; 3H-13	H-8	30.32	3H-12; 3H-13	H-8
14	159.27			160.40		2H-1'	160.87		2H-1'
2'	-	-	-	-	-	-	35.95	2H-1'; 3H-5'	3H-4'
3'	-	-	-	25.64	3H-4'; 3H-5'	2H-1'	-	-	-
CH_2									
2	35.88	H-3a		36.01			36.01		
3	30.10	2H-2		30.19	2H-2		30.19	2H-2	
1'	-	-	-	49.13	2H-2'	H-14	56.91		H-14; 3H-5'
2'	-	-	-	39.47	2H-1'	3H-14'; 3H-5'	-	-	-
3'	-	-	-	-	-	-	26.80	3H-4'	2H-1'; 3H-5'
CH_3									
12	22.84			22.65	H-11		22.65	H-11	
13	22.41			22.23	H-11		22.23	H-11	
15	19.53		H-3a	19.23			19.23		
4'	-			22.28			11.38		
5'	-			22.28			17.13	H-2'	2H-1'
H_2N	-			-			-		
HN				-			-		

[a] Number of hydrogens bound to carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{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 (Table 1) and ^{1}H-^{1}H-COSY spectra.

on a Perkin Elmer model 343 Digital Polarimeter using $CHCl_3$ as solvent. CC was carried out over alumina (activity II-III, 70-230 mesh ASTM) using different mixtures of chloroform and methanol gradient. The alkaloids were purified by repeated PTLC (1 mm thick, 20 x 20 cm Si gel PF_{254} plates) and recycling HPLC using Shimadzu Chromatograph in reverse phase (ODS, Shim-Pack (H), 5 μm, MeOH, 3.0 mL min^{-1}).

Plant material

Croton muscicapa Muell. Arg. was collected in the "caatinga" vegetation near the municipality of Caicó, State of Rio Grande do Norte, Brazil in April 2002. A voucher specimen (AGRA 5995) is deposited in the Herbarium Prof. Lauro Pires Xavier (JPB), Universidade Federal da Paraíba, Brazil.

Extraction and isolation

The dried and powdered roots (2 kg) of *C. muscicapa* were extracted at room temperature using EtOH 95%, furnishing, after solvent evaporation under vacuum, 73.5 g of extract. This extract was dissolved in 3% HCl, filtered over celite and extracted with $CHCl_3$. The aqueous phase was alkalinized with NH_4OH and extracted again with $CHCl_3$ furnishing 3.2 g of a residue. The residue was chromatographed on a neutral aluminum oxide column with a gradient of MeOH in $CHCl_3$ yielding seventy fractions. Alkaloid **4** (583 mg) was purified from fractions 5-8 (1.0 g) after rechromatography on silica gel PTLC using $CHCl_3$:MeOH (99.5:0.5). Fractions 42-49 (15.0 mg) were purified by recycling HPLC using reverse phase with MeOH as solvent to give alkaloid **2** (2.1 mg) and alkaloid **3** (1.7 mg). Alkaloid **1** (5.8 mg) was purified from the fractions 67-70 (15.0 mg) using a silica gel column and a gradient of MeOH in $CHCl_3$ as eluent. Alkaloid **1** was acetylated with acetic anhydride in the presence of pyridine (1:2) overnight at room temperature to furnish the monoacetate **1a** (2.3 mg).

*Muscicapine A (**1**)*

$C_{15}H_{19}NO_2$; red oil; $[\alpha]^{20}_D$ – 44° ($CHCl_3$, *c* 0.05); IR

(KBr) ν_{max}/cm^{-1} 3366, 2932, 2833 and 1650); EIMS (70 eV) *m/z* (rel. int.): 245 (73, M$^{+\bullet}$), 230 (29), 202 (100), 174 (14), 91 (12), 79 (11), 77 (16), 53 (13); NMR data (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC) are given in Tables 1 and 2.

*Muscicapine A acetate (**1a**)*

Acetylation of **1** (4 mg) with acetic anhydride (0.3 mL) in pyridine (0.5 mL) and work-up in the usual way afforded **1a** (3.1 mg) as a yellow oil. NMR data (^{1}H and ^{13}C NMR) are given in Table 1.

*Muscicapine B (**2**)*

$C_{20}H_{29}NO_2$; yellow oil; $[\alpha]^{20}_D$ – 6° ($CHCl_3$, *c* 0.02); EIMS (70 eV) *m/z* (rel. int.): 315 (87, M$^{+\bullet}$), 300 (45), 287 (15), 273 (18), 272 (100), 258 (38), 244 (42), 230 (20), 216 (13), 202 (27), 187 (13), 55 (13), 43 (69), 41 (43); NMR data (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC) are given in Tables 1 and 2.

*Muscicapine C (**3**)*

$C_{20}H_{29}NO_2$; yellow oil; $[\alpha]^{20}_D$ – 9° ($CHCl_3$, *c* 0.05); EIMS (70 eV) *m/z* (rel. int.): 315 (79, M$^{+\bullet}$), 300 (41), 273 (18), 272 (100), 258 (8), 245 (18), 244 (94), 230 (16), 228 (12), 216 (13), 202 (18), 187 (20), 91 (13), 77 (12), 55 (15), 43 (850, 41 (49); NMR data (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC) are given in Tables 1 and 2.

Acknowledgments

The authors are grateful to the Programa de Apoio ao Desenvolvimento Científico e Tecnológico (PADCT)/ Financiadora de Estudos e Projetos (FINEP), Instituto do Milênio do Semi-Árido (IMSEAR), CNPq and FAPERJ for grants, and to CNPq for research fellowships.

References

1. Magalhães, A. F.; Tozzi, A. M. G. A.; Magalhães, E. G.; Moraes, V. R. D.; *Phytother. Res.* **2000**, *14*, 156.
2. Southon, I. W.; Buckingham, J.; *Dictionary of Alkaloids*, Chapman and Hall: London, 1989.
3. Araújo-Júnior, V. T.; Navarro, P. A.; Silva, M. S.; Cunha, E. V. L.; Agra, M. F.; Gray, A. I.; Barbosa-Filho, J. M.; *Ciencia* **2002**, *10*, 286.
4. Araújo-Júnior, V. T.; Silva, M. S.; Cunha, E. V. L.; Agra, M. F.; Silva-Filho, R. N.; Barbosa-Filho, J. M.; Braz-Filho, R.; *Pharm. Biol.* **2004**, 42, 62.
5. Barbosa-Filho, J. M.; Cunha, E. V. L.; Cornélio, M. L.; Dias, C. S.; Gray, A. I.; *Phytochemistry* **1997**, *44*, 959.
6. Büchi, G.; Goldman, I. M.; Mayo, D. W.; *J. Am. Chem. Soc.* **1966**, *88*, 3109.
7. Yang, C. M.; Tanner D. D.; *Can. J. Chem.* **1997**, *75*, 616.
8. Silverstein, R. M.; Webster, F. X.; *Spectrometric Identification of Organic Compounds*, 6th ed., John Wiley & Sons, Inc.: New York, 1998.

Received: July 8, 2003
Published on the web: March 9, 2005