J. Braz. Chem. Soc., Vol. 21, No. 8, 1581-1586, 2010.
Printed in Brazil - ©2010 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Short Report

Diterpene and other Constituents from *Stemodia maritima* (Scrophulariaceae)

Francisco E. A. Rodrigues,[a] Jefferson Q. Lima,[a,b] Maria da Conceição F. de Oliveira,[a] Jackson N. Vasconcelos,[a] Gilvandete M. P. Santiago,[a,c] Jair Mafezoli,[a] Raimundo Braz-Filho[d] and Angela M. C. Arriaga*[*,a]

[a]Curso de Pós-Graduação em Química, Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, CP 6036, 60451-970 Fortaleza-CE, Brazil

[b]Curso de Engenharia Ambiental, Instituto Federal do Ceará, Campus Juazeiro do Norte, 63040-000 Juazeiro do Norte-CE, Brazil

[c]Departamento de Farmácia, Universidade Federal do Ceará, 60430-370 Fortaleza-CE, Brazil

[d]Centro de Ciências e Tecnologias, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 28013-603 Campos dos Goytacazes-RJ, Brazil

Um novo diterpeno, (5*S**,8*S**,9*R**,10*S**)-11β,12β-epoxi-9α-hidróxi-19(4→3)*abeo*-abieta-3,13-dieno-19,18-olideo, e as substâncias conhecidas estemodina, D-manitol, ácido betulínico, uma mistura de 3β-*O*-β-D-glicopiranosil-β-sitosterol e 3β-*O*-β-D-glicopiranosilestigmasterol, e 5,7,4'-triidróxi-3,8,3'-trimetoxiflavona, foram isolados das folhas e talos de *Stemodia maritima.* A elucidação estrutural de todas as substâncias baseou-se na interpretação de dados espectrais, principalmente RMN (1D e 2D) e espectrometria de massa (EM), envolvendo comparação com valores descritos na literatura.

A new diterpene, (5*S**,8*S**,9*R**,10*S**)-11β,12β-epoxy-9α-hydroxy-19(4→3)*abeo*-abieta-3,13-diene-19,18-olide, together with the known compounds stemodin, D-mannitol, betulinic acid, a mixture of 3β-*O*-β-D-glucopyranosyl-β-sitosterol and 3β-*O*-β-D-glucopyranosylstigmasterol and 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone were isolated from the leaves and stems of *Stemodia maritima*. Structural elucidation of all compounds was based on interpretation of spectral data, mainly NMR (1D and 2D) and MS, including comparison with values described in the literature.

Keywords: *Stemodia maritima*, Scrophulariaceae, diterpenes, steroids, flavonoids

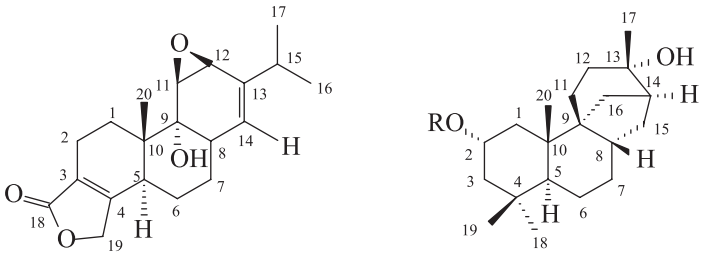
Introduction

Stemodia Benth. is one of Scrophulariaceae genus and occurs in tropical and subtropical regions of the world.[1] Although *Stemodia* comprises about 40 species, the chemical investigation of this genus is restricted to five species[4] from which flavonoids,[2,3] labdane diterpenes[4,5] and diterpenes derivatives with a rare tetracyclic skeletal, named stemodane, were isolated. This later class of diterpenes seems to be chemomarkers of *Stemodia*.[6]

S. maritima Linn. is a very common shrub that widely grows in Northeast Region of Brazil, near the sea coast, where it is known as "melosa". It has been used to treat stomachache, dropsy and swelling by local population, although toxic symptoms was reported in cattle.[7] Stemodane diterpenes, including glycosides derivatives, possessing antiviral and cytotoxic properties were isolated from this species.[6,8-10] The chemical composition and larvicidal activity of its essential oil were recently reported.[11]

On the course of the phytochemical investigation of *S. maritima* from the Northeast Region of Brazil, herein we report the non-volatile composition of this species. A new diterpene, (5*S**,8*S**,9*R**,10*S**)-11β,12β-epoxy-9α-hydroxy-19(4→3)*abeo*-abieta-3,13-diene-19,18-olide (**1**), together with the known compounds stemodin (**2**) (Figure 1), D-mannitol, betulinic acid, a mixture of 3β-*O*-β-D-glucopyranosyl-β-sitosterol and 3β-*O*-β-D-glucopyranosylstigmasterol, and 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone were isolated from the leaves and stems of this plant. Structural elucidation of all compounds was

*e-mail: angelamcarriaga@yahoo.com.br



1 **2** R=H **2a** R=Ac

Figure 1. Compounds **1** e **2** isolated from *Stemodia maritima*.

based on the interpretation of spectral data, meanly NMR (1D and 2D) and MS, and comparison with literature data.

Results and Discussion

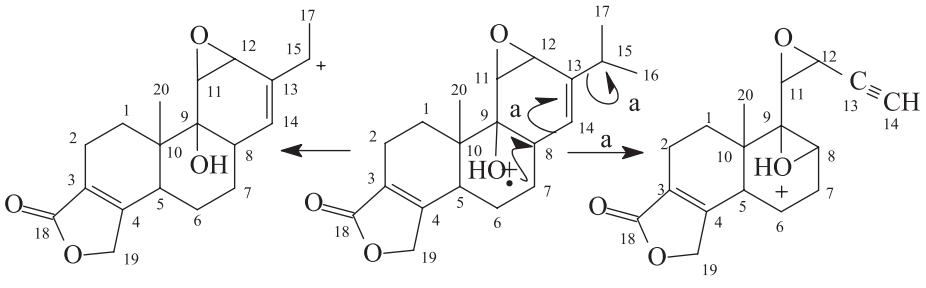
The molecular formula of compound **1** was established through HR-ESI-MS, which showed the quasi-molecular ion peak at *m/z* 331.1799 ([M+1]$^+$, corresponding to the molecular formula $C_{20}H_{26}O_4$ and indicating eight degrees of unsaturation. EIMS from **1** showed the molecular ion peak at *m/z* 330 ($C_{20}H_{26}O_4$, 5%) and additional peaks at *m/z* 315 ($C_{19}H_{23}O_4$, 7%) and *m/z* 287 [$C_{17}H_{19}O_4$, 100%], attributed to fragments **1a** and **1b**, respectively (Figure 2). The presence of a hydroxyl absorption (ν_{max} 3433 cm^{-1}) and an α,β-unsatured-γ-lactone (ν_{max} 1729 cm^{-1}) was inferred from its IR spectrum.

The ^{1}H NMR spectrum (Table 1) revealed the presence of an isopropyl group (δ_H 1.03, d, *J* 6.8 Hz, 3H-16; δ_H 1.05, d, *J* 6.8 Hz, 3H-17; δ_H 2.62, sep, *J* 6.8 Hz, H-15), a methyl group at δ_H 1.01 (3H, s, 3H-20) attached to quaternary carbon, two oxygenated methine hydrogens at δ_H 3.66 (dd, *J* 2.5 and 1.9 Hz, H-11) and δ_H 4.40 (brs, H-12), compatible with the presence of an epoxy ring, two deshielded hydrogen from a oxygenated methylene group at δ_H 4.72 (brdd, *J* 17.2 and 1.6 Hz, H-19α) and δ_H 4.68 (brdd, *J* 17.2 and 1.6 Hz, H-19β), and an olefinic hydrogen at δ_H 5.24 (brd, *J* 5.0 Hz, H-14).

Analysis of BB and DEPT 135° ^{13}C NMR spectra (Table 1) revealed 20 lines, in accordance with the molecular formula $C_{20}H_{26}O_4$. From these data it is possible to deduce the presence of the six non-protonated carbons: one carbonyl group (δ_C 173.9), three sp^2 carbons, one oxygenated sp^3 carbon and one non-oxygenated sp^3 carbon. Additionally, it was observed six methine carbons, including two sp^3 oxygenated at δ_C 66.6 and 59.7 and one sp^2 at δ_C 121.8; five methylene carbons, one of them oxygenated at δ_C 70.4, and three methyl carbons.

The aforementioned data were coherent with a non aromatic abietane-type diterpene that displays an epoxy ring, a tertiary hydroxyl group, an α,β-unsaturated-γ-lactone system and two double bonds, having some similarities with the diterpene triptolide.[12]

The location of these functions in the abietane skeleton was deduced through additional HMBC analysis (Table 1), which revealed the following long-range correlations: the epoxy hydrogens at δ_H 3.66 (H-11) with C-13 (δ_C 140.1, ^3J) and at δ_H 4.4 (H-12) with C-13 (δ_C 140.1, ^2J) and C-14 (δ_C 121.8, ^3J); the isopropyl hydrogen at δ_H 2.62 (H-15,) with C-13 (δ_C 140.1, ^2J) and C-12 (δ_C 66.6, ^3J); the olefin hydrogen at δ_H 5.24 with C-12 (δ_C 66.6, ^3J), C-13 (δ_C 140.1, ^2J) and C-15 (δ_C 28.6, ^3J). The position of the hydroxyl group at C-9 was established based in the correlations of this oxymethine carbon (δ_C 67.9) with the hydrogen of the methyl group (3H-20, δ_H 1.01, ^3J), which



1a *m/z* 315 (39%, M-Me$^\cdot$) **1** **1b** *m/z* 287 (100%, M-Me$_2$CH$^\bullet$)

Figure 2. Fragments postulated to justify some of principal peaks observed in EIMS of **1**.

Table 1. ^{1}H and ^{13}C NMR data assignments for the compound **1** ($CDCl_3$, 500/125 MHz)

	HSQC		HMBC			
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$		
3	125.3	-	2H-2	H-1a, 2H-19		
4	162.0	-	2H-19	2H-6		
9	67.9	-	H-8, H-11	2H-1, 2H-7; H-14, 3H-20		
10	37.0	-	2H-1, 3H-20	2H-6, H-11		
13	140.1	-	H-12, H-14, H-15	H-8, H-11, 3H-16, 3H-17		
18	173.9	-	-	2H-19	^1H-^1H-NOESY	
CH					H	nOe
5	44.2	2.51 (m)	2H-6	2H-1, 2H-7, 3H-20	H-5α	H-1α, H-6α; H-7α, H-19α
8	34.6	2.86 (dd, 12.2, 5.0)	2H-7	2H-6, H-14	H-8β	H-6β, H-7β, 3H-20β
11	59.7	3.66 (dd, 2.5, 1.9)		H-8	H-11α	2H-1
12	66.6	4.40 (brs)	H-11	H-14, H-15	H-12α	H-15, 3H-26, 3H-27
14	121.8	5.24 (brd, 5.0)	H-8	2H-7, H-12, 2H-7	H-14	2H-7, H-8β, H-15, 3H-26, 3H-27
15	28.6	2.62 (sep, 6.8)	3H-16, 3H-17	H-14	-	-
CH_2					-	-
1	28.4	α 1.77 (dd, 12.8, 5.3) β 1.36 (m)	-	3H-20	-	-
2	17.7	2.38 (m) 2.20 (m)	2H-1	-	-	-
6	22.7	α 1.67 (m) β 1.62 (m)	2H-7	-	-	-
7	32.9	β 2.11 (m) α 1.07 (m)	2H-6, H-8	-	-	-
19	70.4	4.72 (brdd, 17.2, 1.6) 4.68 (brdd, 17.2, 1.6)	-	-	-	-
CH_3					-	-
16	22.9	1.03 (d, 6.8)	H-15	3H-17	-	-
17	20.9	1.05 (d, 6.8)	H-15	3H-16	-	-
20	14.0	1.01 (s)		2H-1	3H-20	H-1β, H-2β, H-6β, H-8β

is generally present in abietane-type diterpenoids.[13] Finally, the butenolide ring involving the carbons C-3, C-4, C-18 and C-19 was located by the correlations of the methylene hydrogens at δ_H 4.72 and 4.68 (2H-19) with C-4 (δ_C 162.0, ^2J), C-3 (δ_C 125.3, ^3J) and C-18 (δ_C 173.9, ^3J).

The relative configuration of **1** (Figure 3) was assigned by the analysis of the ^1H-^1H-NOESY spectrum. The β-orientation of the epoxy function (11,12β-epoxide) was determined by the dipolar interactions of the hydrogen at δ_H 3.66 (H-11) with 2H-1 (δ_H 1.77 and 1.36). In addition, the methyl signal at δ_H 1.01 (3H-20) exhibited cross-peaks with the hydrogens at δ_H 2.86 (H-8), δ_H 2.20 (H-2β) and δ_H 1.62 (H-6β). The hydrogen at δ_H 2.51 (H-5) showed dipolar interaction with the hydrogens at δ_H 1.77 (H-1α), 1.67 (H-6α) and 1.07 (H-7α). Based on these correlations, the hydroxyl group at C-9 was established at α position (Figure 3). Therefore, all these data allowed to establish the structure of **1** as (5*S**,8*S**,9*R**,10*S**)-11β,12β-epoxy-9α-hydroxy-19(4→3)*abeo*-abieta-3,13-diene-19,18-olide.

Compound **2** was obtained as colorless crystal and its molecular formula $C_{20}H_{34}O_2$ was deduced by EIMS ($[M]^+$, *m/z* 306) and ^1H and ^{13}C NMR analysis. Its IR spectrum showed hydroxyl absorption at ν_{max} 3311 cm^{-1}. All spectral data were in accordance with the structure of the stemodin (**2**), a stemodane-type diterpene previously isolated from *Stemodia* species.[6,8]

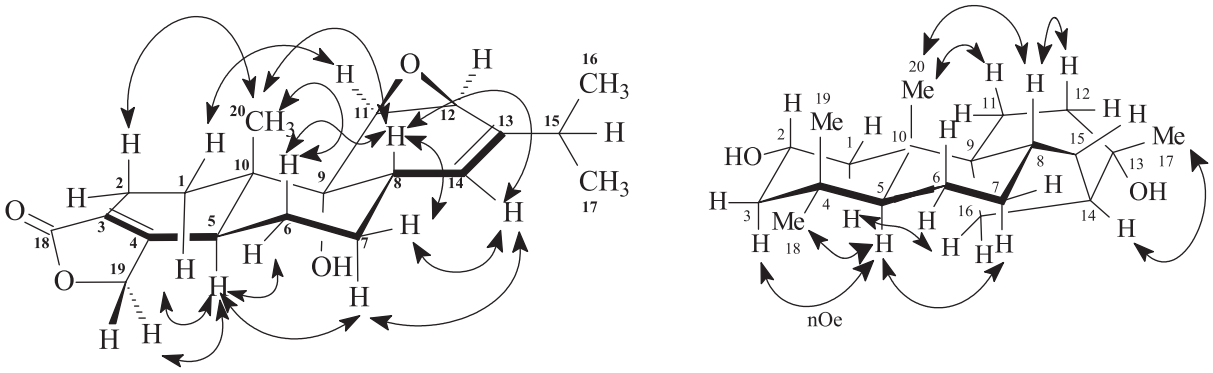


Figure 3. Selected NOESY correlations (depicted by double arrows) for compounds **1** e **2**.

Compound **2** was submitted to acetylation with pyridine and acetic anhydride (see Experimental section), yielding **2a**[8] (Figure 1). The 1D and 2D NMR spectral data of **2** and of its acetyl derivative (**2a**) were also used to complete ^{1}H and ^{13}C chemical shifts described in Table 2. Dipolar interactions observed from ^{1}H-^{1}H-NOESY analysis of **2** are summarized in Figure 3.

The other isolated compounds were identified on the basis of their spectral analysis and comparison with the literature data.

Table 2. ^{1}H and ^{13}C NMR data assignments for the compounds **2** and **2a** ($CDCl_3$, 500/125 MHz)

	2a				**2**	
C	δ_C	δ_H	$^{2}J_{CH}$	$^{3}J_{CH}$	δ_C	δ_H
4	34.9	-	3H-18, 3H-19	-	35.0	-
9	50.3	-	2H-11, 2H-16, 3H-20	H-1b, H-14	50.3	-
10	40.3	-	2H-1, 3H-20	H-6a	40.4	-
13	72.6	-	H-12b, H-14, 3H20	2H-15, 2H-16	72.6	-
AcO	170.8	-	-	H-2	-	-
CH						
2	69.4	4.91 (tt, 11.8, 3.8)	2H-1, 2H-3	3H-18, 3H-19	65.5	3.77 (tt, 11.9, 3.7)
5	46.8	1.24	-	2H-3, 3H-18, 3H-19	46.7	1.24
8	37.0	1.72	-	2H-16	37.0	1.76
14	46.3	1.97	2H-15, 2H-16	3H-17	46.3	1.96
CH_2						
1	41.9	2.00, 1.28	H-2	2H-3	46.0	1.99, 1.21
3	46.7	1.72, 1.08	H-2	2H-1, H-5	50.9	1.78, 1.09 (t, 11.9)
6	22.1	1.40, 1.18	2H-7		22.2	1.42, 1.21
7	36.5	1.92, 1.72	2H-6, H-8		36.6	1.92, 1.15
11	27.9	1.57, 1.40	H-12a	H-16a	27.9	1.65, 1.40
12	33.0	1.52, 1.32	H-11a	H-14, 3H-17	33.0	1.57, 1.43
15	38.2	1.70, 1.25	H-14, 2H-15		38.3	1.74, 1.26
16	30.2	1.80 (d, 11.9), 1.70	-	2H-11, H-15a	30.2	1.82 (brd, 11.6), 1.74
CH_3						
17	28.3	1.12 (s)	-	H-14	28.3	1.13 (s)
18	34.7	0.96 (s)	-	2H-3, H-5, 3H-19	34.8	0.96 (s)
19	23.7	0.95 (s)	-	2H-3, H-5, 3H-18	23.9	0.93 (s)
20	19.5	1.05 (s)	-	2H-1	19.8	0.99 (s)
AcO	21.7	2.02 (s)	-	-	-	-

Experimental

General experimental procedures

Melting points were obtained from a Mettler FP82HT apparatus and are uncorrected. IR spectra were recorded using a Perkin Elmer 1000 FT-IR spectrophotometer. Optical rotations were measured on a Perkin Elmer 341 polarimeter. High resolution electrospray ionization mass spectra (ESI-MS/MS), in positive mode, was performed on a QTOF Micromass spectrometer (QqTOF, Micromass-UK). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C); chemical shifts are given in ppm relative to residual $CHCl_3$ (7.27 and 77.23 ppm). Silica Gel 60 (Merck, 230-400 mesh) was used for analytical TLC. Silica gel 60 (Merck, 60 F_{254}, 0.2 mm) was used for column chromatography. All compounds were visualized on TLC by spraying with vanillin/perchloric acid/EtOH followed by heating.

Plant material

S. maritima was collected during the flowering stage in September 2006 along the Flexeiras Beach, Ceara Cost, Northeast of Brazil. The plant was identified by Dr. F. S. Cavalcanti and Prof. E. P. Nunes from the Herbário Prisco Bezerra (EAC), Universidade Federal do Ceará, Fortaleza, Brazil, where a voucher specimen (# 38483) is deposited.

Extraction and isolation

The fresh stems (200.0 g) of *S. maritima* were exhaustively extracted with ethanol, at room temperature, to obtain a crude material, composed by a precipitate, which was recrystalized from methanol to give D-mannitol[14] (80.0 mg, 0.04%).

The aqueous extract obtained after the essential oil extraction (hydrodistillation) of the fresh stems of *S. maritima* was submitted to liquid-liquid partition with hexane/MeOH (3:7). The hexane fraction (340.0 g) was submitted to column chromatography on silica gel column, using a gradient solvent system of hexane and CH_2Cl_2. Chromatography of the subfraction hexane (380.0 mg) using hexane/EtOAc mixtures with increasing polarity yielded betulinic acid[15] (8.5 mg, 0.0025%). Successive flash chromatography of CH_2Cl_2 subfraction (2.0 g) using 0-100% CH_2Cl_2/EtOAc provided a mixture of 3β-*O*-β-D-glucopyranosyl-β-sitosterol and 3β-*O*-β-D-glucopyranosylstigmasterol[16] (8.2 mg, 0.0024%).

After extraction of the essential oils from the leaves of *S. maritime* by hydrodistillation, the aqueous extract was subjected to liquid-liquid partition with ethyl acetate. The organic fraction (4.0 g) was chromatographed over silica gel with $CHCl_3$, EtOAc and MeOH to afford three subfractions F1-F3. Successive flash column chromatography of F1 (1.2 g), previously eluted from $CHCl_3$, yielded **2** (45.3 mg, 1.13%) after elution with $CHCl_3$/hexane 7:3. From these same column, fraction $CHCl_3$/hexane 9:1 (180.0 mg) was also obtained and rechromatographed over silica gel using the same eluent system to afforded 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone[17] (6.5 mg, 0.0019%) and **1** (15.6 mg, 0.39%).

(5S,8S*,9R*,10S*)-11β,12β-epoxy-9α-hydroxy-19(4→3) abeo-abieta-3,13-diene-19,18-olide (**1**)*

Crystalline Solid; mp 264.6-266.5 °C; IR (film, KBr) ν_{max}/ cm^{-1}: 3433, 2962, 2866, 1729, 1663, 1453, 1344, 1036; HREIMS, *m/z* 331.1799, required *m/z* 331.1909; $[\alpha]_D^{25} = -12.9^{\circ}$ (*c* 1.0, $CHCl_3$).

*Stemodin (**2**)*

Crystalline Solid; mp 189.9-192.4 °C; IR (film, KBr) ν_{max}/ cm^{-1}: 3311, 2954, 1463,1367, 1217,1032; EIMS, *m/z* 306 ($M^{+\cdot}$), 291, 288, 273, 232, 217, 161, 94.

The structures of known compounds were established by 1D ^{1}H and ^{13}C ({^{1}H} and DEPT) and 2D ^{1}H-^{1}H-COSY, HSQC and HMBC NMR spectral data (Table 2) and by comparison of their spectroscopy data with those reported in the literature.[6]

Acetylation of **2**

To a solution of compound **2** (24.0 mg) in pyridine (0.5 mL) were added Ac_2O (1.0 mL) and catalytic amount of DMAP. The mixture was stirred for 5 h at room temperature. Subsequent workup afforded a residue that was chromatographed using hexane/$CHCl_3$ (1:1), hexane/$CHCl_3$ (1:3) as eluent to yield compound **2a**[8] (12.0 mg, 50.0%) as a colorless solid.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

Acknowledgments

The authors acknowledge the financial support provided by Brazilian Agencies CNPq, CAPES, FUNCAP and PRONEX, and are grateful to Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ-RJ-Brazil) for the fellowship to visitant research and grants and to the Centro Nordestino de Aplicação e Uso da Ressonância

Magnética Nuclear (CENAUREMN), Universidade Federal do Ceará (UFC), Fortaleza-CE, for the support to perform of the NMR (1D and 2D) spectra and to the Laboratório Thomson de Espectrometria de Massas, IQ-Unicamp, Campinas-SP, Brazil, for the high resolution mass spectra.

References

1. Chamy, M. C.; Piovano, M.; Garbarino, J. A.; Gambaro, V.; *Phytochemistry* **1991**, *30*, 1719.
2. Ramesh, P.; Nair, A. G. R.; Subramanian, S. S.; *Curr. Sci.* **1979**, *48*, 67.
3. Rodrigues, F. E. A.; *MSc Dissertation*, Universidade Federal do Ceará, Brazil, 2008.
4. Da Silva, W. M. B.; Assunção, J. C. da C.; Araújo, R. M.; Silveira, E. R.; Pessoa, O. D. L.; *J. Braz. Chem. Soc*. **2009**, *20*, 37.
5. Da Silva, L. L. D.; Nascimento, M. S.; Cavalheiro, A. J.; Silveira, D. H. S.; Castro-Gamboa, I.; Furlan, M.; Bolzani, V. S.; *J. Nat. Prod.* **2008**, *71*, 1291.
6. Hufford, C. D.; Oguntimein, B. O.; Muhammad, I.; *J. Nat. Prod.* **1992**, *55*, 48.
7. Silva, D. M.; Correa, F. R.; Medeiros, R. M. T.; Oliveira, O. F.; *Pesquisa Vet. Brasil.* **2006**, *26*, 223.
8. Manchand, P. S.; White, J. D.; Wright, H.; Clardy, J.; *J. Am. Chem. Soc.* **1973**, *95*, 2705.
9. Martin, G. D. A.; Reynolds, W. F.; Reese, P. B.; *Phytochemistry* **2005**, *66*, 901.
10. Hufford, C. D.; Badria, F. A.; Abau-Karam, M.; Shier, W. T.; Rogers, R. D.; *J. Nat. Prod.* **1991**, *54*, 1543.
11. Arriaga, A. M. C.; Rodrigues, F. E. A.; Lemos, T. L. G.; de Oliveira, M. C. F.; Lima, J. Q.; Santiago, G. M. P.; Braz-Filho, R.; Mafezoli, J.; *Nat. Prod. Commun.* **2007**, *2*, 1237.
12. Ghisalberti, E. L.; *Fitoterapia* **1997**, *68*, 303.
13. Duan, H.; Kawazoe, K.; Bando, M.; Kido, M.; Takaishi, Y.; *Phytochemistry* **1997**, *46*, 535.
14. Pouchert, C. J.; Behnke, J.; *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra,* Aldrich Chemical Company: Milwakee, 1993.
15. Kolak, U.; *Turk. J. Chem.* **2007**, *31*, 363.
16. Costa, H. N. R.; Santos, M. C.; Alcântara, A. F. C.; Silva, M. C.; França, R. C.; Piló-Veloso, D.; *Quim. Nova* **2008**, *31*, 744.
17. Roitman, J. N.; James, L. F.; *Phytochemistry* **1985**, *24*, 835.

Received: June 24, 2009
Web Release Date: March 25, 2010