Acetylcholinesterase Inhibitory Pyridine Alkaloids of the Leaves of *Senna multijuga*[⊥]

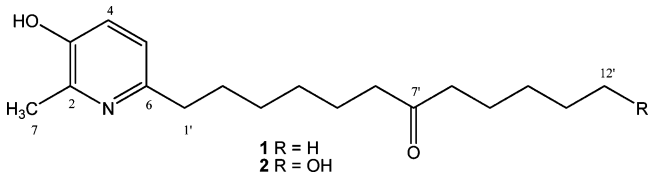
Maria A. R. Serrano,[†] Marcos Pivatto,*[,†] Welington Francisco,[†] Amanda Danuello,[†] Luis O. Regasini,[†] Elaine M. C. Lopes,[‡] Marcia N. Lopes,[†] Maria C. M. Young,[‡] and Vanderlan S. Bolzani*[,†]

São Paulo State University (UNESP), P.O. Box 355, 14801-970, Araraquara, SP, Brazil, and Organic Chemistry Department, Institute of Chemistry, Nuclei for Bioassays, Biosynthesis and Ecophysiology of Natural Products (NuBBE), and Section of Plant Biochemistry and Physiology, Institute of Botany (IBt), P.O. Box 4009, 01061-970, São Paulo, SP, Brazil

Received October 12, 2009

Two unusual pyridine alkaloids, 7′-multijuguinone (**1**) and 12′-hydroxy-7′-multijuguinone (**2**), were isolated from the leaves of *Senna multijuga*, together with the known flavonoid rutin. The structures of the new alkaloids were established on the basis of spectroscopic data interpretation. Compounds **1** and **2** exhibited moderate in vitro acetylcholinesterase (AChE) inhibitory activity, in comparison with the standard compound physostigmine.

Senna multijuga (Rich.) Irwin et Barneby (syn. *Cassia multijuga* Rich.) (Fabaceae) is a medium-size evergreen tree distributed widely in several Brazilian regions and other biomes from Africa.[1] Several chemical studies of seeds, leaves, and roots have shown the occurrence of flavonoids,[2] anthraquinones,[3,4] polysaccharides,[5] and chromones.[6] In Brazil, this species is popularly known as "pau-cigarra", "caquera", "topeiuia", "aleluia", and "canafístula" and is used as an ornamental plant in several regions of the country due to its attractive yellow flowers.[1,7] Although some species of the *Senna* (*Cassia*) genus have been reported to be an important source of phenolic compounds with diverse biological and pharmacological properties,[8] only the seeds of *S. multijuga* are used for the treatment of ophthalmic and skin infections.[2] However, no phytochemical study on the isolation of the secondary metabolites of this species has been conducted previously. As part of our ongoing research on Atlantic Forest plant species,[9] we selected *S. multijuga* for study. From an ethanolic extract of the leaves were isolated two new pyridine alkaloids, 7′-multijuguinone (**1**) and 12′-hydroxy-7′-multijuguinone (**2**), together with the known flavonoid rutin.[10] The occurrence of 2-methyl-3-hydroxy-6-*n*-alkyl pyridine alkaloids is unusual, and only one report has been described in the literature.[11] Herein, we present the isolation, structure elucidation, and acetylcholinesterase-inhibitory properties of **1** and **2** by bioautography and microplate screening assays.



The first Dragendorff-positive compound, 7′-multijuguinone (**1**), was isolated as a white solid, mp 103−105 °C. The molecular weight of **1** was measured by HRESITOFMS, and the molecular formula was established as $C_{18}H_{29}NO_2$, with a hydrogen deficiency index of 5. The observed pseudomolecular ion peak at *m/z* 292.2271 $[M + H]^+$ was calculated for $C_{18}H_{30}NO_2$, 292.2271. The UV spectrum, with absorption maxima at 222 and 287 nm, suggested the presence of a ketone and substituted aromatic moiety chromophores, respectively. IR absorption bands were assigned to hydroxy (3401 cm^{-1}), carbonyl (1701 cm^{-1}), and pyridine ring (1578, 1498, and 1464 cm^{-1}) functional groups. Examination of the ^1H, ^{13}C, and DEPT NMR spectra recorded in $CDCl_3$ (Table 1) showed the presence of a 2,3,6-trisubstituted pyridine ring [δ 7.00 (1H, d, $J = 8.0$ Hz, H-4), 6.84 (1H, d, $J = 8.0$ Hz, H-5); δ 145.3 (C-2), 148.8 (C-3), 122.7 (C-4), 120.8 (C-5), and 152.9 (C-6)], two methyl groups at δ 0.88 (t, H-12′, $J = 7.5$ Hz, δ_C 13.9) and 2.47 (s, H-7, δ_C 18.5), and 10 methylenes at δ 2.67 (t, H-1′, $J = 8.0$ Hz, δ_C 37.0), 1.64 (qt, H-2′, $J = 8.0$ Hz, δ_C 30.2), 1.30 (m, H-3′, H-4′, and H-11′, δ_C 29.1, 29.0, and 22.4), 1.55 (qt, H-5′ and H-9′, $J = 7.5$ Hz, δ_C 23.8 and 23.5), 2.36 (t, H-6′, $J = 7.5$ Hz, δ_C 42.7), 2.37 (t, H-8′, $J = 7.5$ Hz, δ_C 42.8), and 1.27 (m, H-10′, δ_C 31.4) and suggested the presence of a long linear side chain in the structure of **1**. Furthermore, the side chain from C-1′ to C-12′ was deduced as being attached at C-6 of the pyridine nucleus by a gHMBC NMR experiment. Three-bond correlations were observed from H-5 to C-1′, H-1′ to C-5, and H-2′ to C-6 (Figure 1), supporting a 2-methyl-3-hydroxy-6-*n*-alkyl pyridine substitution pattern. The 7′-oxododecyl unit linked at C-6 is different from the long-chain pattern observed for other piperidine and pyridine alkaloids from *Senna* (*Cassia*) species. The position of the 7′-oxo group on the straight side chain was also defined by gHMBC data analysis, for which the main correlations are represented in Figure 1. Furthermore, in a TOCSY experiment irradiation of H-1′ and H-12′ showed

Table 1. NMR Spectroscopic Data for **1** and **2** in $CDCl_3$[a]

	1		**2**	
position	δ_C (mult.)	δ_H[b] (mult., *J* in Hz)	δ_C (mult.)	δ_H[b] (mult., *J* in Hz)
2	145.3 (C)		145.4 (C)	
3	148.8 (C)		148.8 (C)	
4	122.7 (CH)	7.00 d (8.0)	122.6 (CH_2)	7.02 d (8.0)
5	120.8 (CH)	6.84 d (8.0)	120.7 (CH_2)	6.85 d (8.0)
6	152.9 (C)		152.7 (C)	
7	18.5 (CH_3)	2.47 s	18.6 (CH_3)	2.48 s
1′	37.0 (CH_2)	2.67 t (8.0)	37.0 (CH_2)	2.68 t (7.5)
2′	30.2 (CH_2)	1.64 qt (8.0)	30.1 (CH_2)	1.66 qt (7.5)
3′	29.1 (CH_2)	1.30 m	29.7 (CH_2)	1.28 m
4′	29.0 (CH_2)	1.30 m	28.9 (CH_2)	1.33 m
5′	23.8 (CH_2)	1.55 qt (7.5)	23.7 (CH_2)	1.59 qt (7.5)
6′	42.7 (CH_2)	2.36 t (7.5)	42.6 (CH_2)	2.38 t (7.5)
7′	211.9 (C)		211.7 (C)	
8′	42.8 (CH_2)	2.37 t (7.5)	42.7 (CH_2)	2.41 t (7.5)
9′	23.5 (CH_2)	1.55 qt (7.5)	23.4 (CH_2)	1.61 qt (7.5)
10′	31.4 (CH_2)	1.27 m	25.3 (CH_2)	1.39 m
11′	22.4 (CH_2)	1.30 m	32.3 (CH_2)	1.59 qt (6.5)
12′	13.9 (CH_3)	0.88 t (7.5)	62.5 (CH_2)	3.67 t (6.5)

[a] Recorded at 500 and 125 MHz for ^1H and ^{13}C NMR, respectively. [b] Multiplicity of signals is given as follows: s, singlet; d, doublet; t, triplet; qt, quintuplet; m, multiplet.

[⊥] Dedicated to the late Dr. John W. Daly of NIDDK, NIH, Bethesda, Maryland, and the late Dr. Richard E. Moore of the University of Hawaii at Manoa for their pioneering work on bioactive natural products.

* To whom correspondences should be addressed. Tel: +55 (16) 3301-6660. Fax: +55 (16) 3322-7932. E-mail: bolzaniv@iq.unesp.br.

[†] São Paulo State University, UNESP.

[‡] Institute of Botany.

10.1021/np900644x
Published on Web 12/14/2009

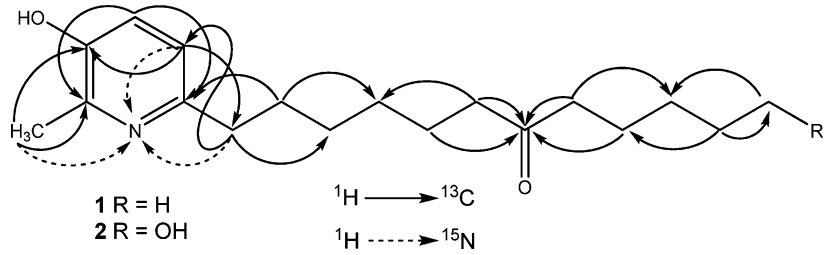


Figure 1. Key gHMBC correlations for **1** and **2**.

correlations with H-2′−H-6′ and H-8′−H-12′, respectively. HOMODEC experiments were carried out decoupling H-2′ (δ 1.64), which enabled the loss of multiplicity from the neighboring H-1′ and H-3′ (overlapped with H-4′). When H-5′ was decoupled, loss of multiplicity was observed for H-6′ and H-4′ (overlapped with H-3′), supporting the same chemical shift for H-3′ and H-4′. The same pattern was observed with H-10′ and H-11′ when H-9′ and H-12′ were decoupled. The interpretation of these gHMBC sequence cross-peaks led to the establishment of the oxo function at the C-7′ position in the side chain. Furthermore, the gHMBC correlations of H-5 with C-3 (^3J) and C-1′ (^3J), together with that from H-7 to C-2 (^2J) and C-3 (^3J), allowed the hydroxy group to be located at C-3, which was consistent with a previous study on bioactive piperidine alkaloids from *Senna* (*Cassia*) species, with features similar to **1**.[9] Additionally, the gHMBC (^{1}H−^{15}N) spectrum showed a correlation between the nitrogen signal at δ 308.8 and signals at δ 2.67 (H-1′), 6.84 (H-5), and 2.47 (H-7). All this evidence agreed with the hydroxy group being located at C-3 and confirmed the assumption made on the substitution pattern in pyridine alkaloid **1**, which must be 2-methyl-3-hydroxy-6-*n*-(7′-oxododecyl) pyridine, similar to those found for piperidine alkaloids from other *Senna* (*Cassia*) species, except for the ketone group position in the side chain. These results suggested that the hydroxy group of this pyridine alkaloid is at C-3, which is not in agreement with those previously reported for a similar pyridine alkaloid from this species, with no hydroxy group being located at C-5.[5]

Compound **2** was obtained as a white solid, mp 71−73 °C, and gave a positive test with Dragendorff reagent. This compound showed spectroscopic features very similar to those of **1**. The HRESITOFMS for **2** exhibited a pseudomolecular ion peak at *m/z* 308.2226 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_3$, 308.2220), appropriate for the molecular formula $C_{18}H_{29}NO_3$, with a hydrogen deficiency index of 5 and being 16 mass units more than that of **1**. The IR spectrum revealed the carbonyl absorption band at 1703 cm^{-1}, a pyridine ring at 1584, 1489, and 1464 cm^{-1}, and a hydroxy group band at 3392 cm^{-1}. The ^{1}H and ^{13}C NMR spectra of compound **2** showed signals very close to those of **1** (Table 1), as in the ^{1}H NMR spectrum, which demonstrates the presence of a substituted pyridine ring system at δ 7.02 (1H, d, J = 8.0 Hz, H-4) and 6.85 (1H, d, J = 8.0 Hz, H-5). Additionally, the methyl hydrogens of CH_3-7 at δ 2.48, analyzed together with ^{13}C NMR data, confirmed that the pyridine moiety is similar to that of **1**. However, differences were evident between these compounds since the methyl group (CH_3-12′) located in the side chain of **1** was replaced with a hydroxy methylene group in **2** at δ 3.67 (2H, t, J = 6.5, H-12′, δ_C 62.5). These data, supported by the ^{13}C NMR, DEPT, gCOSY, gHMQC, gHMBC, TOCSY, and HOMODEC spectra, were in agreement with a pyridine system bearing one hydroxy group, one methyl, and one long-chain substituent. According to these data, the structure of **2** was determined as 2-methyl-3-hydroxy-6-*n*-(7′-oxo-12′-hydroxydodecyl)pyridine. All compounds isolated were screened for their AChE inhibitory activity, using bioautography and microplate tests, and the results are shown in Table 2. The preliminary TLC assay suggested moderate activity for compounds **1** and **2**, which showed the minimum amounts of these alkaloids required for AChE inhibition of 0.75 and 0.18 μg, respectively, when compared with physostigmine (positive control, 0.05 μg). Rutin proved to be inactive, since the minimum amount required to show activity was over 100 μg. In addition, the microplate test confirmed activity of the compounds **1** and **2**, which exhibited weak AChE inhibition of 13% and 30% at 350 mM.

Table 2. AChE Inhibitory Activity of Compounds **1** and **2** and Rutin

	AChE inhibitory activity	
compound	bioautography[a]	microplate[b]
1	0.75	13 ± 2.0
2	0.18	30 ± 1.3
rutin	>100	not tested
physostigmine[c]	0.05	87 ± 0.1

[a] Minimum amount required for inhibition of AChE on TLC plates (in μg). [b] Percentage of AChE inhibition at 350 mM. [c] Positive control.

Experimental Section

General Experimental Procedures. Melting points were recorded on a digital Microquimica MQAPF-302 apparatus and are uncorrected. UV data were acquired using an Amersham Ultrospec 2100 pro UV/vis spectrophotometer. IR spectra were recorded on a Nicolet iS10 FT-IR spectrometer coupled with an ATR accessory (the samples were pressed against a crystal of Ge). The 1D (^{1}H, ^{13}C, DEPT, NOESY, TOCSY, and HOMODEC) and 2D (^{1}H−^{1}H gCOSY, gHMQC, gHMBC, and ^{1}H−^{15}N gHMBC) NMR experiments were recorded on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 30 °C and referenced using TMS (δ_{TMS} = 0.00) as internal standard or residual solvent resonances of $CDCl_3$ at δ 7.26 and 77.0 ppm, respectively, for ^{1}H and ^{13}C. High-resolution mass spectra with electrospray ionization were measured on an ultrOTOF$_Q$ (Bruker Daltonics) operating in positive mode. MeOH−H_2O (4:1) was used as solvent system.

TLC was performed on silica gel F_{254} plates (0.20 mm, Fluka), and spots were visualized under UV light (254 and 366 nm) and spraying with Dragendorff's reagent or anisaldehyde−H_2SO_4, followed by charring for 5 min. Preparative TLC was carried out with Analtech 500 μm thick silica gel GF plates (20 × 20 cm, Uniplate) and visualized under UV light at 366 nm. Analytical HPLC separation was performed on a Varian (Palo Alto, CA) ProStar system equipped with a ternary pump model 340, a photodiode array detector model 330, and an autosampler model 410, controlled by Star chromatography workstation version 5.52 software. Preparative HPLC separation was performed on a Varian PrepStar system equipped with a UV−vis detector model 320 and a binary pump model SD-1 manual injector, controlled by Star chromatography workstation version 5.51 software. The columns used were a Phenomenex Luna C_{18} (250 × 4.60 mm, 5 μm) and a preparative Phenomenex Luna C_{18} (250 × 21.20 mm, 10 μm) protected by corresponding guard columns. All solvents used in the experimental procedures were distilled previously. For chromatographic analysis, HPLC-grade solvents (J. T. Baker, Xalostoc, Mexico) were employed. Water was purified immediately prior to use with a Milli Q plus system (Millipore, Milford, MA).

Plant Material. Leaves of *S. multijuga* were collected in Araraquara (São Paulo, Brazil) in July 2008 by one of the authors (M.P.). The plant was identified by Inês Cordeiro from the Institute of Botany in São Paulo-SP, Brazil. A voucher specimen (SP 384103) has been deposited in the herbarium of this institute.

Extraction and Isolation. The dried and powdered leaves (2.7 kg) were extracted with ethanol (8 L × 4) for five days at room temperature.

The solvent was removed under reduced pressure by rotary evaporation, yielding a thick syrup (210 g). The crude ethanol extract (100 g) was further reconstituted in MeOH−H_2O (4:1), filtered, and then partitioned with *n*-hexane (250 mL × 5), CH_2Cl_2 (250 mL × 4), EtOAc (250 mL × 4), and *n*-BuOH (250 mL × 2), successively. All phases were concentrated under reduced pressure. The CH_2Cl_2 fraction (15.9 g) was dissolved in 500 mL of 5% HCl aqueous solution (ca. pH 2), filtered, and partitioned with EtOAc (50 mL × 3) to remove nonbasic components. Then, NH_4OH (30%) was added to the aqueous phase until ca. pH 8.5 and partitioned with CH_2Cl_2 (50 mL × 5). The latter organic layer was washed with H_2O (100 mL × 3) and concentrated in order to give a neutral alkaloidal fraction (457 mg), which was purified by using preparative TLC with *n*-hexane−CH_2Cl_2−EtOAc (1.5:2.0:6.5) as developing solvent (×2). The bands with R_f values of 0.50 and 0.18 were scraped and eluted with MeOH, affording pure compounds **1** (49.1 mg) and **2** (60.5 mg). Fraction EtOAc (2.20 g) was subjected to column chromatography on RP-C_{18} and eluted with MeOH−H_2O (5:95 to 100:0) to yield six fractions (EtOAc-F1 to EtOAc-F6). Fractions EtOAc-F2 and EtOAc-F3 (527 mg) were further purified by preparative HPLC on RP-C_{18} and eluted with MeOH−H_2O (40:60, flow rate 19 mL/min, UV 254 nm), affording compound **3** (t_R 17.4 min, 42.0 mg).

Bioautographic Assay. The AChE inhibitory activity of pure compounds was determined using a TLC bioautographic assay as previously described.[12] The TLC layers were spotted with **1** and **2** in a range from 0.09 to 6.00 μg developed with *n*-hexane−EtOAc (1:4, v/v) and subsequently dried. The plates were then sprayed with the enzyme solution (6.66 U/mL), thoroughly dried, and incubated at 37 °C for 20 min (moist atmosphere). Enzyme activity was detected by spraying with a solution consisting of 0.25% of 1-naphthyl acetate in EtOH plus 0.25% aqueous solution of Fast Blue B salt. Potential acetylcholinesterase inhibitors appeared as clear zones on a purple-colored background. Electric eel AChE type V (product no. C 2888, 1000 U) and the other reagents were purchased from Sigma-Aldrich.

Microplate Assay. The AChE inhibitory activity of compounds **1**−**3** was evaluated using the modified methods of Ellman et al. and Rhee et al. in a 96-well microplate, as previously described.[13] Using this method, the enzyme hydrolyzes the substrate acetylthiocholine, resulting in the production of thiocholine, which reacts with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate and can be detected at 405 nm. To the 96-well plates were added 25 μL of an aqueous solution of acetylthiocholine iodide (15 mM), 125 μL of DTNB (3 mM) in buffer C, 50 μL of buffer B, and 25 μL of sample diluted in buffer A, to give a range of concentrations from 0.78 to 100 μg/mL. The absorbance was measured at 405 nm every 30 s three times. Furthermore, 25 μL of 0.22 U/mL of the enzyme was added and the absorbance was again read every 10 min two times. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the rate of the reaction before the addition of the enzyme from the rate of the enzyme reaction. The percentage of inhibition was calculated in comparison to a blank (10% MeOH in buffer A), and a positive control of physostigmine was used in the same range of concentrations. The following buffers were used: buffer A, 50 mM Tris-HCl (pH 8); buffer B, 50 mM Tris-HCl (pH 8) containing 0.1% bovine serum albumin V fraction; buffer C, 50 mM Tris-HCl (pH 8) containing 0.10 M NaCl and 0.02 M $MgCl_2 \cdot 6H_2O$. All of the reagents were purchased from Sigma-Aldrich.

7′-Multijuguinone (1): white solid; mp 103−105 °C; UV (MeOH) λ_{max} (log ε) 222 (3.83), 287 (3.63) nm; IR ν_{max} 3401, 2919, 2849, 1701, 1578, 1498, 1464, 1285, 1164, 1130, 832, 725 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESITOFMS *m*/*z* 292.2271 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_2$, 292.2271); TLC R_f 0.50 (1.5:2.0:6.5 *n*-hexane−CH_2Cl_2−EtOAc).

12′-Hydroxy-7′-multijuguinone (2): white solid; mp 71−73 °C; UV (MeOH) λ_{max} (log ε) 222 (3.83), 287 (3.63) nm; IR (film) ν_{max} 3392, 2931, 2851, 1703, 1584, 1489, 1464, 1290, 1248, 1160, 1128, 1048, 827, 725 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESITOFMS *m*/*z* 308.2226 $[M + H]^+$ (calcd for $C_{18}H_{30}NO_3$, 308.2220); TLC R_f 0.18 (1.5:2:6.5 *n*-hexane−CH_2Cl_2−EtOAc).

Rutin: yellow solid; spectroscopic data were in agreement with those previously published.[10]

Acknowledgment. This research was supported by grants from the FAPESP as part of Biota-FAPESP, The Biodiversity Virtual Institute Program (www.biota.org.br) grant no. 03/02176-7, awarded to V.S.B. The researchers also acknowledge FAPESP, CAPES and CNPq, for fellowships. The authors thank Dr. N. P. Lopes (USP in Ribeirão Preto-SP, Brazil) for high-resolution mass spectra and Dr. I. Cordeiro (IBt in São Paulo-SP, Brazil) for authentication of plant material.

Supporting Information Available: Copies of high-resolution mass, ^1H and ^{13}C NMR, and selected 2D spectra of 7′-multijuguinone (**1**) and 12′-hydroxy-7′-multijuguinone (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Lorenzi, H. *Árvores Brasileiras: Manual de Identificação e Cultivo de Plantas Arbóreas Nativas do Brasil*; Plantarum: Nova Odessa, 1998; p 166.
(2) Dubey, P.; Gupta, P. C. *Planta Med.* **1980**, *38*, 165–168.
(3) Singh, J. *Planta Med.* **1981**, *41*, 397–399.
(4) Tiwari, R. D.; Singh, J. *Z. Naturforsch.* **1983**, *38b*, 1136–1137.
(5) Rechia, C. G. V.; Sierakowski, M. R.; Ganter, J. L. M. S.; Reicher, F. *Int. J. Biol. Macromol.* **1995**, *17*, 409–412.
(6) Singh, J. *Phytochemistry* **1982**, *21*, 1177–1179.
(7) Di Stasi, L. C.; Hiruma-Lima, C. A. *Plantas Medicinais na Amazônia e na Mata Atlântica*; UNESP: São Paulo, 2002; p 281.
(8) Viegas, C., Jr.; Rezende, A.; Silva, D. H. S.; Castro-Gamboa, I.; Bolzani, V. S.; Barreiro, E. J.; Miranda, A. L. P.; Alexandre-Moreira, M. S.; Young, M. C. M. *Quim. Nova* **2006**, *29*, 1279–1286.
(9) (a) Bolzani, V. S.; Gunatilaka, A. A. L.; Kingston, D.G. I. *Tetrahedron* **1995**, *51*, 5929–5934. (b) Bolzani, V. S.; Young, M. C. M.; Furlan, M.; Cavalheiro, A. J.; Araújo, A. R.; Silva, D. H. S.; Lopes, M. N. *An. Acad. Bras. Cienc.* **1999**, *71*, 181–187. (c) Alexandre-Moreira, M. S.; Viegas, C., Jr.; Miranda, A. L. P.; Bolzani, V. S.; Barreiro, E. J. *Planta Med.* **2003**, *69*, 795–799. (d) Viegas, C., Jr.; Bolzani, V. S.; Furlan, M.; Barreiro, E. J.; Young, M. C. M.; Tomazela, D.; Eberlin, M. N. *J. Nat. Prod.* **2004**, *67*, 908–910. (e) Viegas, C., Jr.; Bolzani, V. S.; Pimentel, L. S. B.; Castro, N. G.; Cabral, R. F.; Costa, R. S.; Floyd, C.; Rocha, M. S.; Young, M. C. M.; Barreiro, E. J.; Fraga, C. A. M. *Bioorg. Med. Chem.* **2005**, *13*, 4184–4190. (f) Viegas, C., Jr.; Bolzani, V. S.; Barreiro, E. J.; Fraga, C. A. M. *Mini-Rev. Med. Chem.* **2005**, *5*, 915–926. (g) Pivatto, M.; Crotti, A. E. M.; Lopes, N. P.; Castro-Gamboa, I.; Rezende, A.; Viegas, C., Jr.; Young, M. C. M.; Furlan, M.; Bolzani, V. S. *J. Braz. Chem. Soc.* **2005**, *16*, 1431–1438. (h) Viegas, C., Jr.; Silva, D. H. S.; Pivatto, M.; Rezende, A.; Castro-Gamboa, I.; Bolzani, V. S.; Nair, M. G. *J. Nat. Prod.* **2007**, *70*, 2026–2028. (i) Viegas, C., Jr.; Alexandre-Moreira, M. S.; Fraga, C. A. M.; Barreiro, E. J.; Bolzani, V. S.; Miranda, A. L. P. *Chem. Pharm. Bull.* **2008**, *56*, 407–412. (j) Castro, N. G.; Costa, R. S.; Pimentel, L. S. B.; Danuello, A.; Romeiro, N. C.; Viegas, C., Jr.; Barreiro, E. J.; Fraga, C. A. M.; Bolzani, V. S.; Rocha, M. S. *Eur. J. Pharmacol.* **2008**, *580*, 339–349.
(10) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389–1397.
(11) Sriphong, L.; Sotanaphun, U.; Limsirichaikul, S.; Wetwitayaklung, P.; Chaichantipyuth, C.; Pummangura, S. *Planta Med.* **2003**, *69*, 1054–1056.
(12) Marston, A.; Kissling, J.; Hostettmann, K. *Phytochem. Anal.* **2002**, *13*, 51–54.
(13) (a) Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88–95. (b) Rhee, I. K.; van de Meent, M.; Ingkaninan, K.; Verpoorte, R. *J. Chromatogr. A* **2001**, *915*, 217–223.

NP900644X